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SYSTEMATIC EMBRYOLOGY OF THE ANISOPHYLLEACEAE[1]

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ABSTRACT

An embryological study of Anisophylleaceae, which comprise *Anisophyllea, Combretocarpus, Poga,* and *Polygonanthus,* and which have traditionally most often been referred as a tribe or subfamily to Rhizophoraceae, is presented as a contribution to the clarification of the systematic position of the family and the evolutionary interrelationships of its constituent genera. The gametic chromosome number of *Combretocarpus* is reported as $n = 8$, that of the other three genera as $n = 7$. Embryologically Anisophylleaceae are diversified and show differences from genus to genus, but they are clearly distinct from Rhizophoraceae in having their combination of consistent character states, including persistent nucellar tissue at least until early stages of seed development, thin two cell-layered inner integument (*Poga* and *Polygonanthus*), and exalbuminous seeds. In contrast to Rhizophoraceae, Anisophylleaceae agree almost completely with Myrtales in their embryological features of the order. Embryological evidence therefore supports the recognition of Anisophylleaceae as a distinct family and, with support from other lines of evidence, suggests a Myrtalean affinity for the family. Proposed assignments of Anisophylleaceae to Rosales or to Cornales are not supported. An analysis of similarities in character states in the four genera suggests that the ancestral Anisophylleaceae diverged into two main branches: one leading to *Anisophyllea* and *Combretocarpus,* and the other leading to *Poga* and *Polygonanthus. Combretocarpus,* with which *Anisophyllea* shares a few synapomorphies, is most specialized within the family in having many apomorphies. In contrast, *Poga* and *Polygonanthus* share many plesiomorphies, most of which are also common to *Anisophyllea.*

Anisophylleaceae, as defined here, consists of four genera and 34 species, *Anisophyllea* (30 spp.), *Combretocarpus* (1 sp.), *Poga* (1 sp.), and *Polygonanthus* (2 spp.) (Airy Shaw, 1973; Cronquist, 1981, 1983). In contrast with the stable assignment of three other genera, various authors have referred *Polygonanthus* to Euphorbiaceae (Ducke, 1932, 1933; Kuhlmann, 1940), Olacaceae (Croizat, 1939), Saxifragaceae (Baehni & Dansereau, 1939), or to its own family, Polygonanthaceae (Croizat, 1943). Despite these, there is little doubt, on the basis of morphological and wood anatom-

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TABLE 1. Studied taxa and collections.

Taxa	Collections
Anisophyllea disticha (Jack.) Baill.	Singapore. Bukit Timah Nature Reserve. *Sidek Bin Kiah & Tan Yam Leong s.n. in 1984*, no voucher. Singapore. Botanic Garden, Singapore. *Sidek Bin Kiah s.n. in 1984*, no voucher; *Mohd Shah s.n. in 1984*, no voucher. Malaysia. Maxwell Hill, Perak. *B. C. Stone 15403*, (KLU, MO). Brunei. *A. M. Juncosa s.n. in 1981*, no voucher.
Anisophyllea sp.	Cameroon. *D. W. Thomas 3494*, (MO).
Combretocarpus rotundatus (Miq.) Dans.	Malaysia. Kuching, Sarawak. *P. Chai s.n. in 1981, 1983, and 1985*, no voucher. Brunei. *A. M. Juncosa s.n. in 1983*, no voucher.
Poga oleosa Pierre	Cameroon. Korup Natl. Park. *D. W. Thomas 2273*, (MO). Nigeria. Awi, Akamkpa. *J. O. Ariwaodo s.n. in 1983*, (FHI 99607).
Polygonanthus amazonicus Ducke	Brazil. Along the Rio Paca, Amazonas. *J. Zarucchi 3138, 3184*, (US).

ical evidence, that *Polygonanthus* fits well in Anisophylleaceae, together with the three other genera that have traditionally been placed there (see Kuhlmann, 1944; Pires & Rodrigues, 1971; Van Vliet, 1976). Of the four genera of this family, *Anisophyllea* is relatively widely distributed in tropical Africa and Asia, also occurring in tropical South America; *Combretocarpus* is restricted to West Malaysia, *Poga* to tropical West Africa; and *Polygonanthus* to the Amazon Basin of Brazil (Pires & Rodrigues, 1971).

The relationships of Anisophylleaceae have been controversial. A traditional view, and the one most widely accepted, is that Anisophylleaceae have close affinities with Rhizophoraceae, and they often have been considered a tribe or subfamily within a broadly conceived Rhizophoraceae (Bentham & Hooker, 1865; Baillon, 1877; Schimper, 1893; Melchior, 1964; Pires & Rodrigues, 1971; Geh & Keng, 1974; Van Vliet, 1976; Takhtajan, 1980). Even when they have been treated as a distinct family, Anisophylleaceae have generally been considered closely related to Rhizophoraceae (Ridley, 1922; Corner, 1940). The resulting family, Rhizophoraceae sensu lato, has traditionally been placed in the Myrtales (Bentham & Hooker, 1865; Schimper, 1893; Melchior, 1964; Takhtajan, 1980), but Thorne (1983) placed Rhizophoraceae (composed of two subfamilies: Rhizophoroideae and Anisophylleoideae) in the Cornales.

Recently, however, Cronquist (1981, 1983) concluded that Anisophylleaceae were not closely related to Rhizophoraceae and assigned them to Rosales (Rosidae) and Rhizophoraceae sensu stricto to its own order, Rhizophorales (Rosidae). Dahlgren (1983), who also denied any close relationships between Anisophylleaceae and Rhizophoraceae, placed Anisophylleaceae in Cornales (Corniflorae) and Rhizophoraceae in its own order, Rhizophorales (Myrtiflorae; see also Dahlgren & Thorne, 1984).

In the light of these diverse opinions, we have attempted to determine whether Anisophylleaceae are actually closely related to Rhizophoraceae sensu stricto or not, or whether they might even be grouped together as one family. If the two groups are not closely related, what are their respective affinities?

Anisophylleaceae have been studied to a very limited extent, particularly regarding their anatomical characteristics. Their wood anatomy, however, has been studied relatively intensively (Marco, 1935; Geh & Keng, 1974; Van Vliet, 1976). Based on a comparison of the wood anatomy of the two groups, Van Vliet (1976) supported a broad definition of Rhizophoraceae including Anisophylleaceae. In contrast, Behnke (1984), basing his conclusions on the features of their sieve-tube plastids, suggested Anisophylleaceae were quite distinct from Rhizophoraceae sensu stricto. He found that both *Anisophyllea* and *Combretocarpus* have S-type plastids (containing starch grains only) in contrast to the P-type plastids (containing protein) that are characteristic of Rhizophoraceae sensu stricto. Another interesting distinction between the two groups, which has been known for some time, is that all four genera of Anisophylleaceae are aluminum accumulators; whereas the genera of Rhizophoraceae sensu stricto are not (Chenery, 1948; Kukachka & Miller, 1980). Although embryological information has been extremely useful in suggesting relationships at this level (see Tobe &

Raven, 1983), almost no information is available on Anisophylleaceae. The only published data on ovule morphology (of "*Anisophylleia zeylanica*") is that of Karsten (1891) nearly 100 years ago; however, most of these observations seem to be incorrect, as we shall discuss subsequently. Vaughan (1970) described the mature seed coat structure of *Poga,* providing a drawing; Geh and Keng (1974) reported on the endosperm in the seeds of *Anisophyllea* and *Combretocarpus.* Except for these fragments of information, apparently nothing has been reported about the embryological features of Anisophylleaceae.

In this paper, we present an overall study of the embryology of Anisophylleaceae, which is intended to provide information bearing on their relationships and systematic position. We have studied *Anisophyllea* and *Combretocarpus* in detail, and *Poga* and *Polygonanthus* to a lesser degree. Important features have been noted for all genera and are presented here.

Materials and Methods

All four genera, *Anisophyllea, Combretocarpus, Poga,* and *Polygonanthus,* were investigated in this study. The species we studied are listed in Table 1 together with their voucher information. Flower buds and fruits in all stages of development were collected and fixed in FAA (5 parts stock formalin; 5 parts glacial acetic acid; 90 parts 70% ethanol); however, female buds of *Poga oleosa* and fruits of *Polygonanthus amazonicus* were not available. Herbarium material of *Anisophyllea* and *Combretocarpus* was studied to supplement our observations of fruits and seeds.

Preparations of microtome sections for observation were made following standard paraffin techniques. After dehydration through a tertiary-butyl alcohol series, the samples were embedded in Paraplast with 56–58°C mp. Flower buds of *Anisophyllea disticha* and fruits of *Poga oleosa* were too hard to be sectioned without being softened initially. Therefore, after these structures were trimmed to expose their tissues, the embedded samples attached to blocks were soaked in a mixture of a 10 : 3 : 90 glycerol : 10% Aerosol OT : water (Schmid & Turner, 1977) for at least several days at 20–25°C and then sectioned. Serial sections 6–10 μm thick were stained with Heidenhain's hematoxylin, safranin, and fast-green FCF and were mounted in Entellan. Mature seed coats of *Anisophyllea* sp. (*D. W. Thomas 3494,* MO) and *Poga oleosa,* which were too thick and hard to be sectioned by standard paraffin techniques, were embedded in a JB-4 plastic and stained with 0.1% Toluidine Blue.

In order to count the number of cells in mature pollen, we attempted to use safranin-staining of the grains (Tobe & Raven, 1984). We failed to obtain any staining of the pollen nuclei, however, probably because a thick exine hinders the infiltration of dye. Consequently, we counted the number of cells in the pollen using microtome-sectioned pollen grains. The expressions we have used for the frequency of different shapes of microspore tetrads follow those of Schmid (1982).

Observations

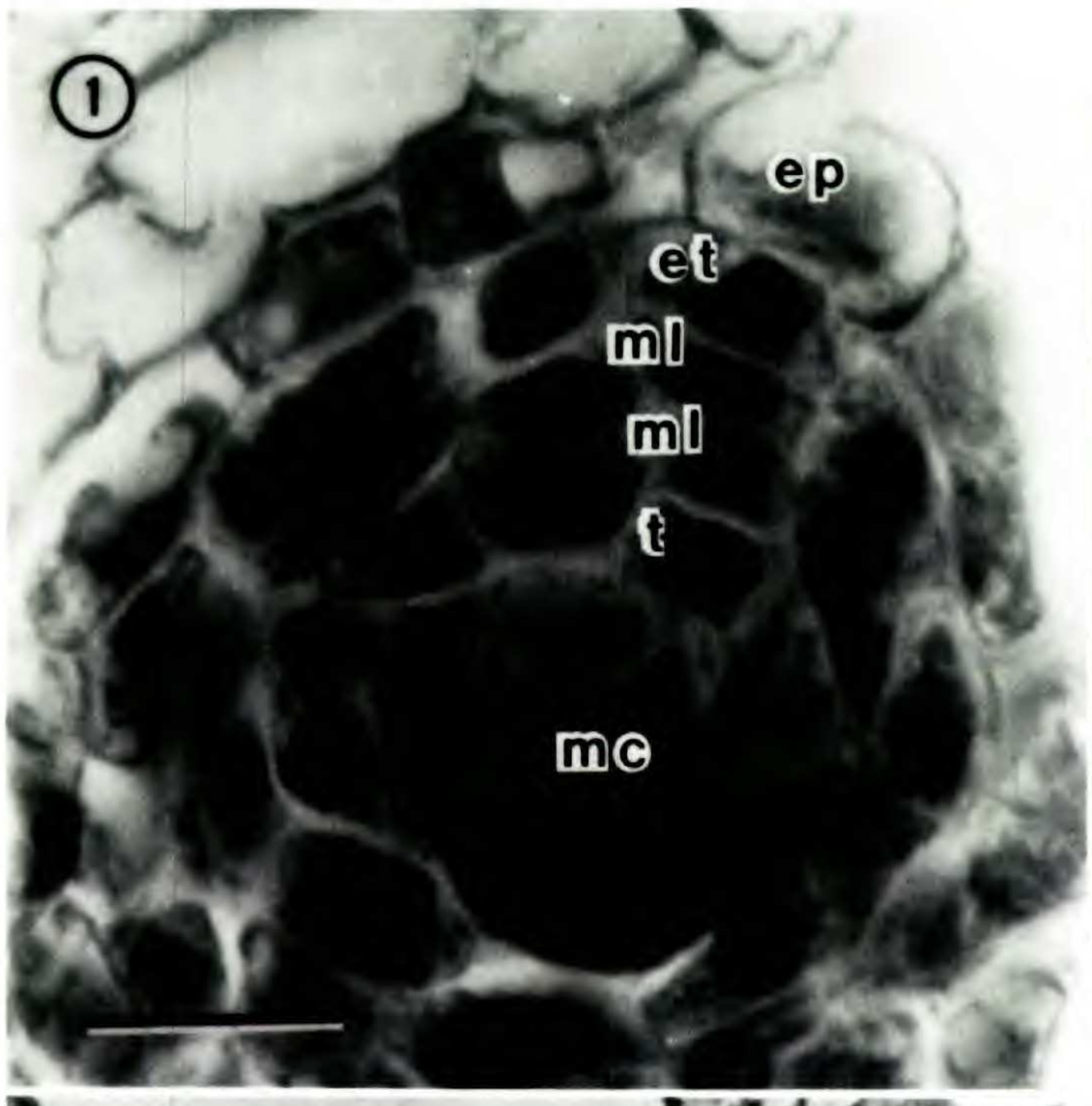
Anisophyllea R. Br.

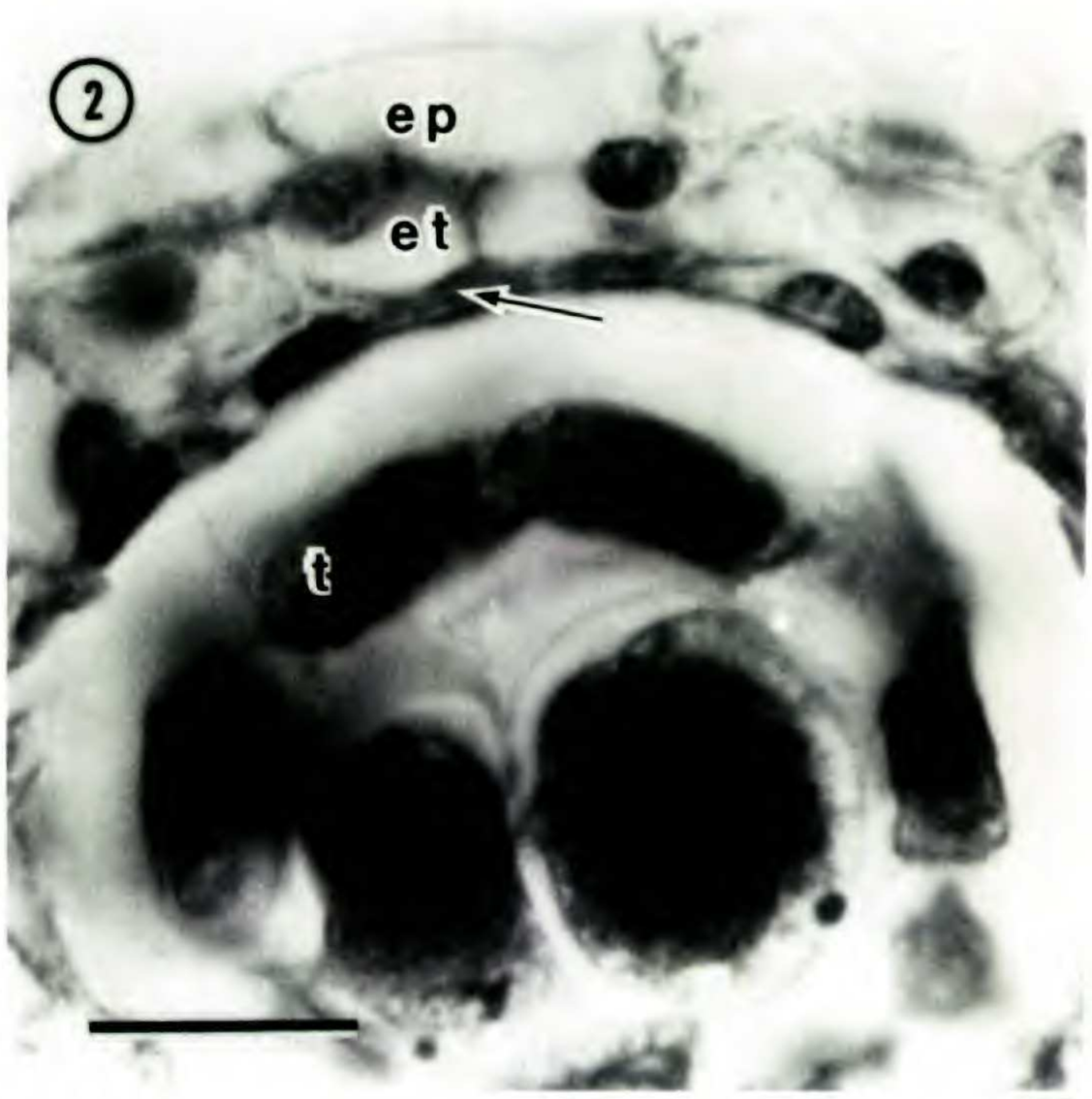
The embryological characteristics were basically the same in the two species studied, one from Africa and one from Asia. The features reported in the following descriptions were found to be common to both species, unless particular comments are given.

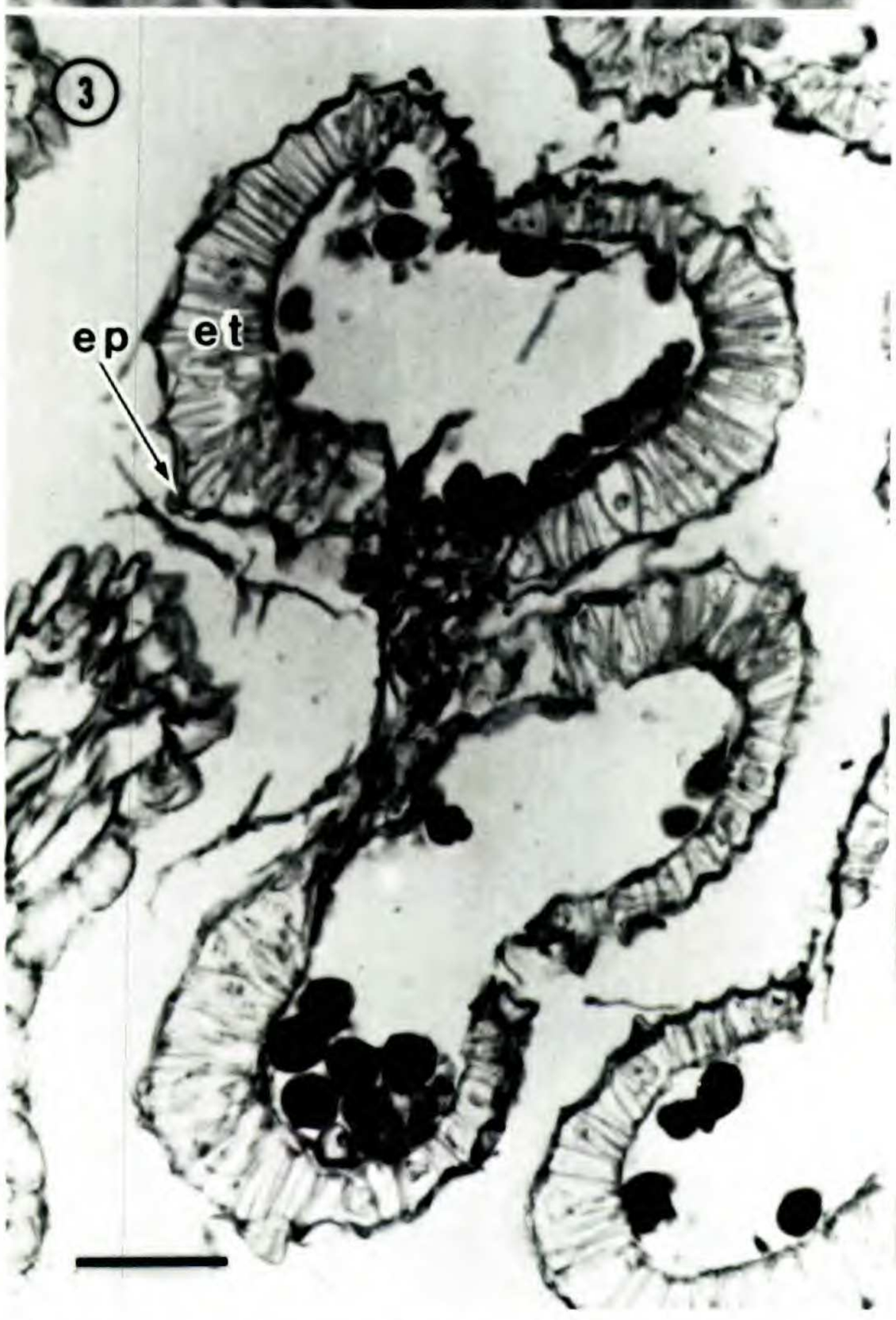
Anther and microspores. The anther is tetrasporangiate. The wall prior to maturation comprises basically five cell layers: an epidermis, an endothecium, two middle layers, and a tapetum (Fig. 1); the wall formation therefore conforms to be the Basic type (Davis, 1966: 10). The anther wall, however, often has only one middle layer, which shares a histogenetic origin with the tapetum. The tapetum is glandular (Fig. 2). At one point in their development, the cells of the tapetum become 2-nucleate, but subsequently the two nuclei fuse with each other. During maturation, the middle layer(s) degenerate and the epidermal cells are stretched tangentially while the cells of the endothecium become more or less enlarged (Fig. 2). Eventually, the endothecium develops fibrous thickenings. Although the epidermis persists, it is often collapsed on the endothecium (Fig. 3). Anther dehiscence takes place by longitudinal slits (Fig. 3). The connective tissue between the two microsporangia of each theca is completely disorganized before an anther dehisces (Fig. 3).

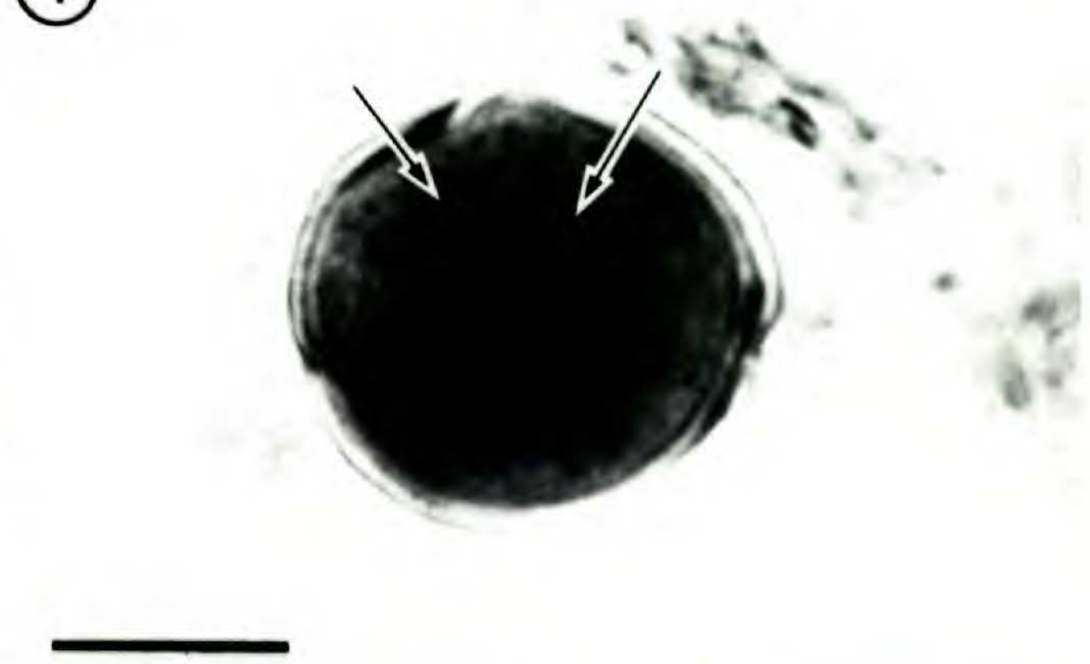
Meiosis in a microspore mother cell is accompanied by simultaneous cytokinesis, and the resultant microspore tetrads, on the basis of 50 selected tetrads (of *Anisophyllea disticha*), are "usually" (92%) tetrahedral, "very occasionally" (6%) decussate, and "rarely" (2%) isobilateral. The pollen grains are two-celled at the time of shedding (Fig. 4).

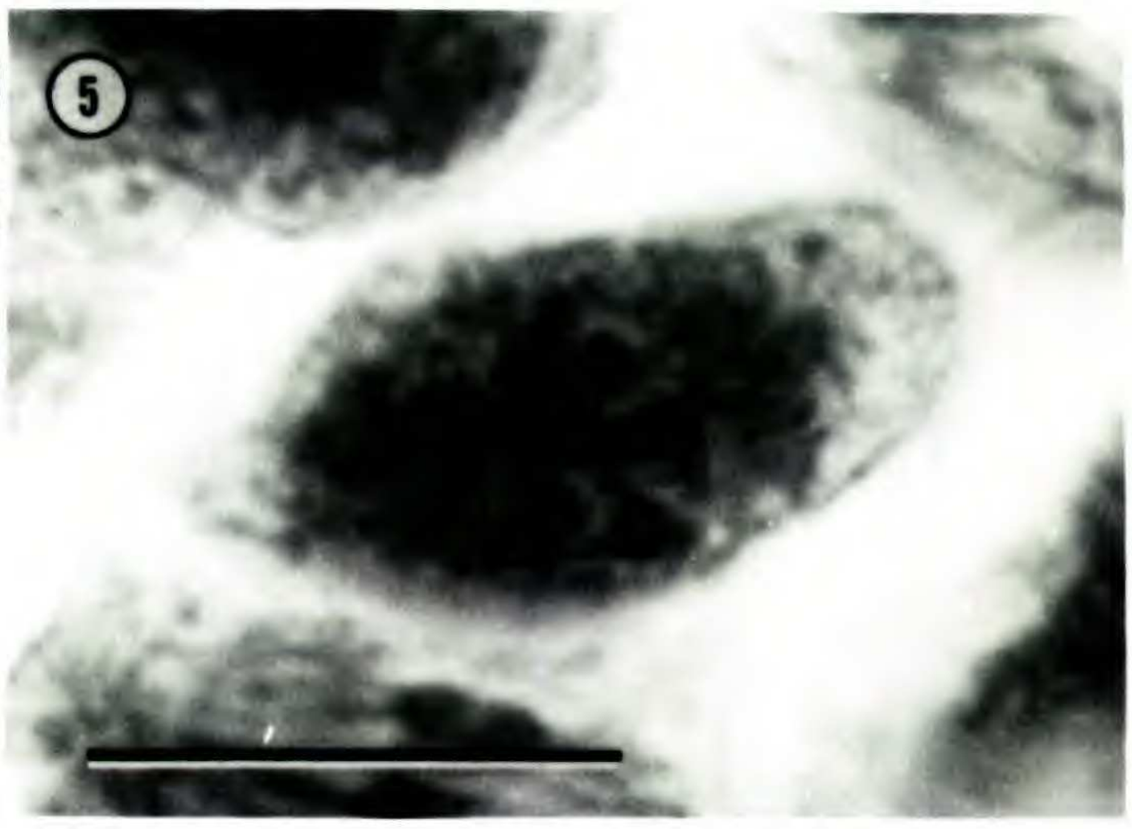
Chromosomes. Since pollen mother cells between the telophase of meiosis I and the meta-











FIGURES 1–5. *Anisophyllea*.—1, 2, 4, 5. *A. disticha*.—3. *A.* sp. (*D. W. Thomas 3494*, MO).—1. Transverse section (TS) of a young anther showing the five cell-layered wall structure. Bar = 10 μm.—2. TS of an older anther with degenerating middle layers (arrow). Bar = 10 μm.—3. TS of a developed anther. Its wall consists of the fibrous endothecium and the epidermis. Bar = 50 μm.—4. Two-celled mature pollen at the time of shedding. Arrows indicate nuclei of the two cells. Bar = 10 μm.—5. Chromosomes of a pollen mother cell at a stage between telophase I and metaphase II. $n = 7$. Bar = 10 μm. ep, epidermis; et, endothecium; ml, middle layer; t, tapetum; mc, microspore mother cell.

phase of meiosis II were fixed by chance and included in microtome sections, we were able to count the chromosome number of *Anisophyllea* for the first time: *A. disticha* has $n = 7$ (Fig. 5).

Megagametophyte and nucellus. The ovule is anatropous. A single archesporial cell differentiates beneath the apical dermal layer of the nucellus (Fig. 6). The archesporial cell divides periclinally into two: the upper primary parietal cell and the lower sporogenous cell (Fig. 7). The primary parietal cell divides periclinally, and its derivatives further divide anticlinally and periclinally, forming parietal tissue with three to five layers above the embryo sac. The sporogenous cell develops into a megaspore mother cell and undergoes meiosis, giving rise to a linear tetrad of megaspores. A triad of megaspores may also be formed by suppression of the second, mitotic division on the micropylar side. In the megaspore tetrad (or triad), the chalazal megaspore functions (Fig. 8). A functional megaspore develops successively into a 2- (Fig. 9), 4- (Fig. 10), and 8-nucleate embryo sac (Fig. 11). Thus the mode of the embryo sac formation is of the *Polygonum* type. The synergids are slightly hooked (Fig. 12). The three antipodal cells are ephemeral, degenerating before fertilization. The two polar nuclei fuse into a single central nucleus, which is positioned near the egg apparatus (Fig. 13). Consequently an organized mature embryo sac has only five nuclei or cells: an egg cell, two synergids, and two polar nuclei (as a single central nucleus; Figs. 12, 13).

During megasporogenesis and megagametogenesis, apical epidermal cells of the nucellus divide periclinally, and their daughter cells also repeat periclinal divisions. As a result, a four to six cell-layered nucellar cap is formed above the embryo sac (Fig. 14); Karsten (1891) also illustrated such a nucellar growth in "*Anisophylleia zeylanica.*" The nucellar cap and the other nucellar tissue, both of which enclose the embryo sac, persist into younger stages of fruit development (Figs. 11, 14, 17). There is no case in which the nucellar tissue degenerates before fertilization so that the embryo sac directly borders on the integument.

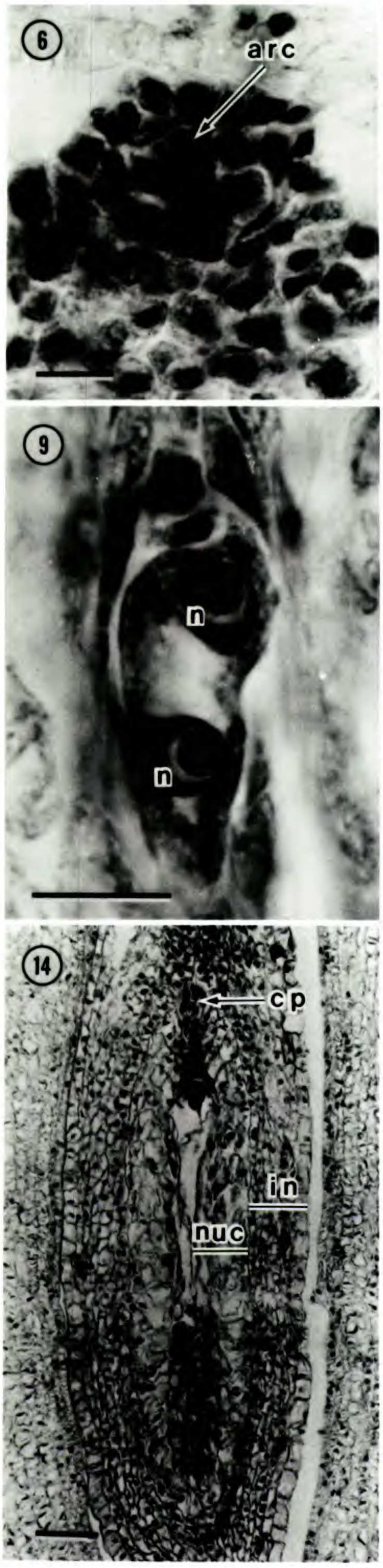
Integument. The ovule has a single integument (Figs. 7, 15), although Karsten (1891) described the ovule of "*Anisophylleia zeylanica*" as having two integuments. Judging from the drawing he published, it seems very probable that Karsten misunderstood a persistent, lateral nucellar tissue surrounding the embryo sac as the inner integument and the (only) true integument as the outer integument.

A micropyle is always formed by the integument, excepting one very unusual case in which the integument did not grow beyond the nucellar apex (Fig. 16). In this respect as well, Karsten (1891) seems to have erred: he considered the persistent, lateral nucellar tissue to be the inner integument, which he concluded did not enclose the nucellar apex. In fact, Karsten concluded that a micropyle is not formed in "*Anisophylleia zeylanica.*" Referring to Karsten's drawing of ovules and descriptions, we also mistakenly characterized the ovule of *Anisophyllea* not only as being bitegmic but also as having a nucellar beak (which actually was the well-developed persistent nucellar cap; see Tobe & Raven, 1983).

The integument is about five to seven cells thick in *Anisophyllea disticha* (Fig. 15) and about four to five cells thick in *A.* sp. (Fig. 17). The thickness of the integument is not different from one part of ovule to another, and therefore the cross section of the ovule is nearly circular (Fig. 17). A raphe bundle ramifies oblique-laterally toward the chalazal end (Fig. 18). Therefore in cross section the ovule or fruit has four to five vascular bundles at the peripheral part of the integument of testa (Fig. 19).

Throughout the development of the ovule or fruit, the integument or seed coat is thickened by secondary multiplication. However, the innermost cell layer never differentiates toward the so-called endothelium.

Fertilization, endosperm, and embryo. Despite their multiovular condition in *Anisophyllea* (usually four and rarely three ovules per ovary), fruits were always one-seeded. Fertilization is porogamous. Endosperm formation is of the Nuclear type (Fig. 20). Because of incompleteness of our fruit sample, we could not confirm whether or not wall formation takes place in free endosperm nuclei. Hand-sectioned mature seeds of the two species we studied lacked endosperm (Fig. 21). Concerning the presence of endosperm in seeds, Hou (1958) described that in *Anisophyllea disticha* seeds consist of a solid body, of which the main part is formed by a thick, hard albumen. Geh and Keng (1974) stated that in *Anisophyllea disticha,* the entire undifferentiated embryo is embedded in endosperm; consequently, they characterized the seed of Anisophylleae (*Anisophyllea* and *Combretocarpus*) as albuminous. Based on results of our observations, however, it seems that what Hou thought



FIGURES 6–14. *Anisophyllea*.—6, 7, 9–13. *A. disticha*.—8. *A.* sp. (*D. W. Thomas 3494*, MO).—6. Longitudinal section (LS) of an ovular primordium with the 1-celled archesporium. Bar = 10 μm.—7. LS of a young ovule with the primary parietal cell. Bar = 10 μm.—8. LS of a young ovule with the functional megaspore. Arrows above the functional megaspore indicate three degenerating megaspores. Bar = 10 μm.—9. LS of an ovule at the 2-nucleate embryo sac stage. Bar = 10 μm.—10. LS of an older ovule at the 4-nucleate embryo sac stage. One of the two nuclei at the micropylar side appears in the next section. Bar = 10 μm.—11. LS of a nearly

to be the thick, hard albumen was actually the embryo itself, and that the seed that Geh and Keng observed was too young to confirm the endosperm condition.

Although we did not pursue the whole process of embryogenesis either, the development of proembryos and embryos seems to proceed normally (Fig. 20). In embryos of the two species we studied, which were dissected from mature fruits, we could not observe differentiation of the cotyledons. Geh and Keng (1974), however, reported two protuberances on the apical part of the embryo in *Anisophyllea disticha,* which they interpreted as two cotyledons. We conclude that the cotyledons of *Anisophyllea* either develop incompletely or are essentially absent in *Anisophyllea.* No hypostase is differentiated after fertilization.

Mature seed and seed coat. The mature seed is narrowly cylindrical, 13.0–13.5 mm long and 3.8–4.0 mm thick in *Anisophyllea disticha,* whereas it is ovoid or elliptical in outline, 13.0–13.8 mm long and 6.0–6.4 mm thick in *A.* sp. (Fig. 21). In the young seed, the seed coat appears to be constructed of a thick, massive tissue, with the outer epidermis specialized and tanniferous (Fig. 22). In the mature seed, the seed coat is formed both of a conspicuous outer epidermis and a multiple inner layer about 25–30 cells thick (Fig. 23). The cells of the outer epidermis are thick-walled and cuboid, whereas those of the underlying multiple inner layer are also thick-walled but extremely stretched tangentially.

COMBRETOCARPUS HOOK F.

Anther and microspores. The anther is tetrasporangiate. The wall prior to maturation comprises five cell layers: an epidermis, endothecium, two middle layers, and a tapetum (Fig. 24). Wall formation conforms to the Basic type. During maturation, the cells of the epidermis are somewhat enlarged and become tanniferous; the cells of the endothecium are also enlarged; the middle layers degenerate (Fig. 25). The tapetum is glandular, and its cells become 2-nucleate. The two nuclei in a tapetal cell later are fused with each other. Thus the mature anther wall is composed of the persistent but somewhat collapsed epidermis and the fibrous endothecium (Fig. 26). By the time of anther dehiscence, the connective tissue between two microsporangia of each teca degenerates completely. After dehiscence by longitudinal slits, the anther wall is remarkably reflexed (Fig. 27).

Meiosis in the microspore mother cells is accompanied by simultaneous cytokinesis. The shape of the resultant tetrads, on the basis of the examination of 50 selected tetrads, is "usually" (78%) tetrahedral, "occasionally" (14%) decussate, and "very occasionally" (8%) isobilateral. The pollen grains are two-celled at the time of shedding (Fig. 28).

Chromosomes. Pollen mother cells at the metaphase of meiosis I happened to be fixed and appeared in microtome sections. On the basis of those sections, we observed the chromosomes of *Combretocarpus* for the first time and determined $n = 8$ (Fig. 29). Size differences seem to be present among those eight chromosomes.

Megagametophyte and nucellus. The ovule is anatropous and crassinucellate. The archesporium is nearly always 1-celled (Fig. 30). A multicellular archesporium may very rarely differentiate—an ovule or young fruit containing twin embryo sacs was very rarely observed (Fig. 38). The archesporial cell divides periclinally into two: the upper primary parietal cell and the lower sporogenous cell. The primary parietal cell may or may not divide further periclinally; if it does so, a two cell-layered parietal tissue is formed. The sporogenous cell develops into a megaspore mother cell (Fig. 31). After enlarging in volume, the megaspore mother cell undergoes meiosis. After meiosis I, however, the subsequent mitosis in each megaspore of the dyad is not accompanied by cytokinesis. As a result, both the micropylar and the chalazal megaspore of the dyad become 2-nucleate (Fig. 32). The chalazal megaspore is functional. Then, while the micropylar megaspore degenerates, the two nuclei in the

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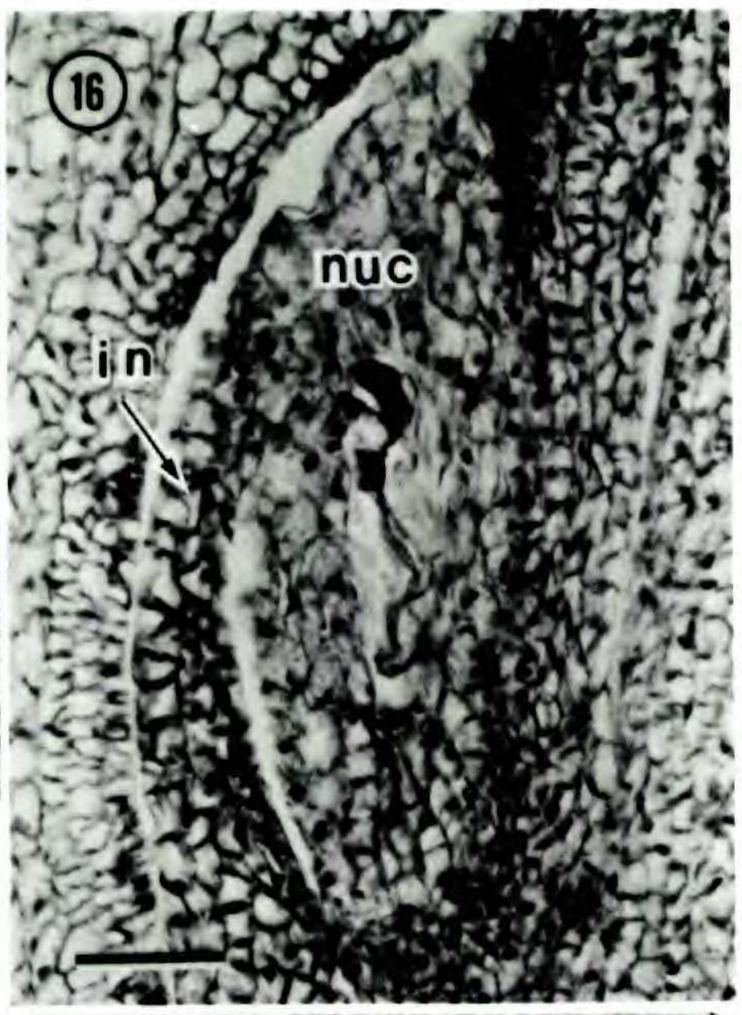
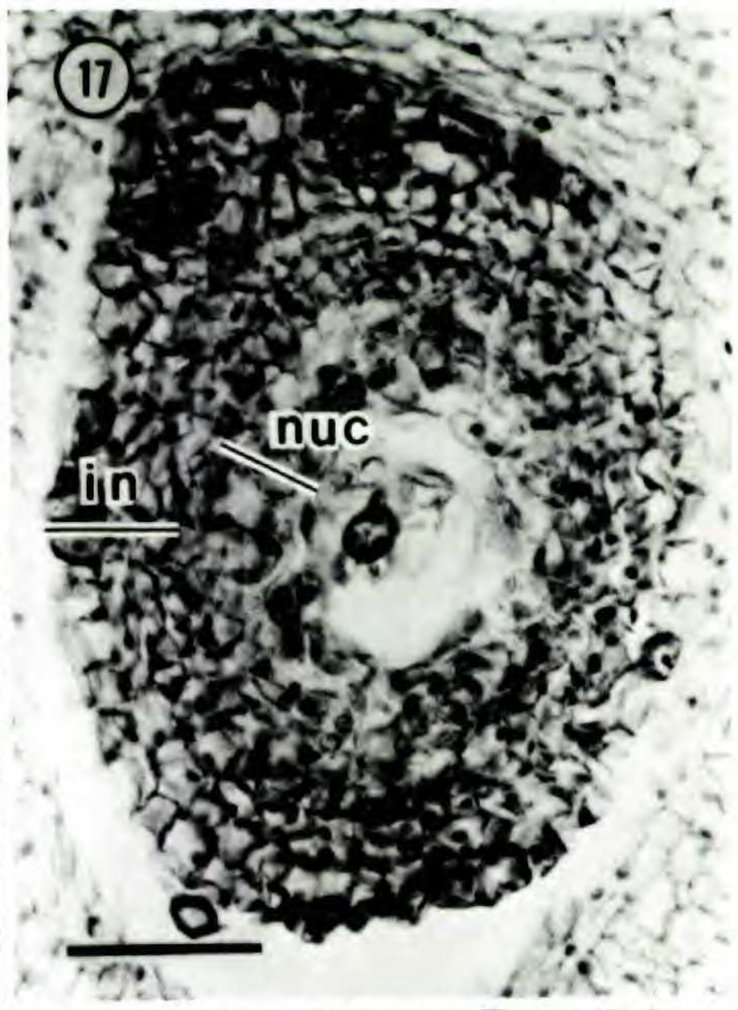
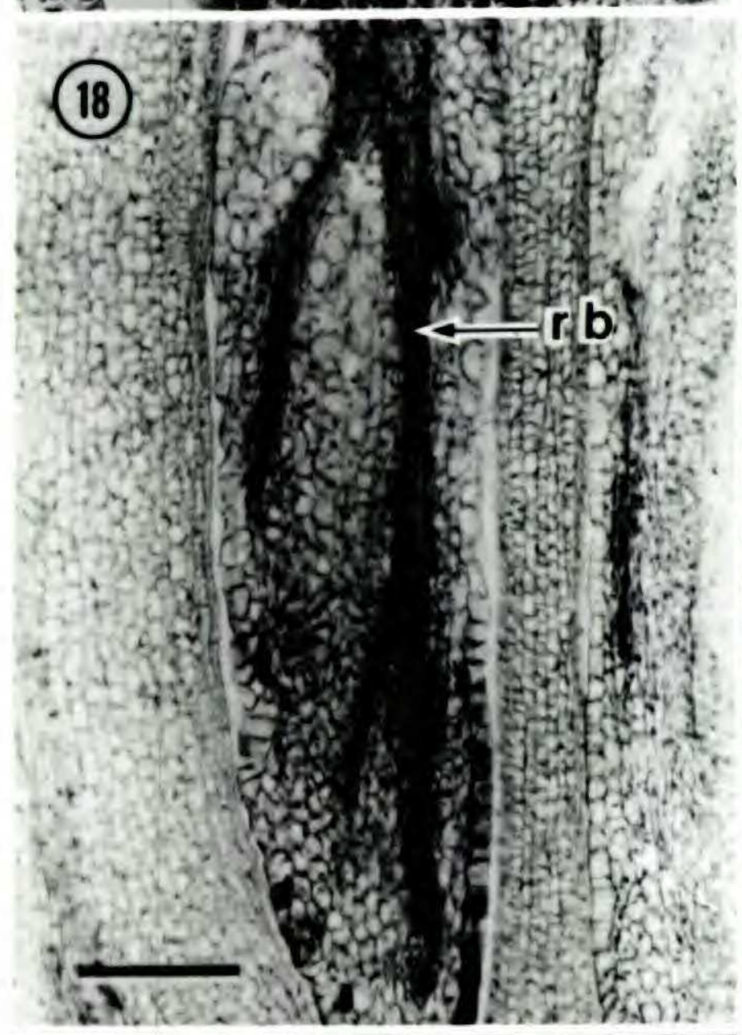
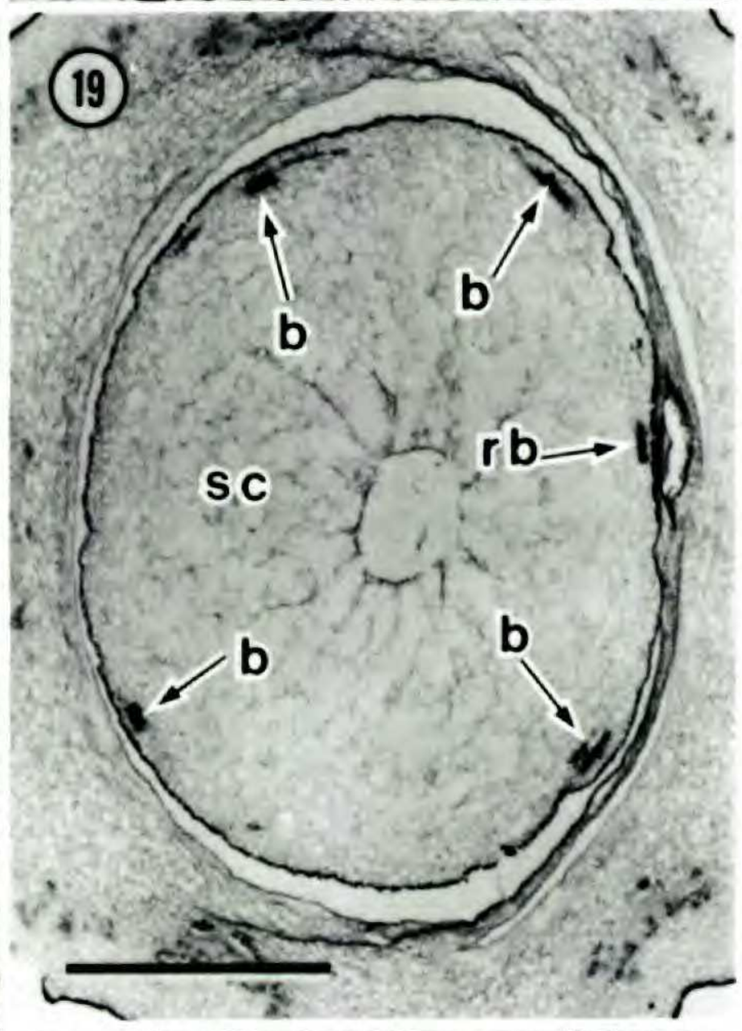
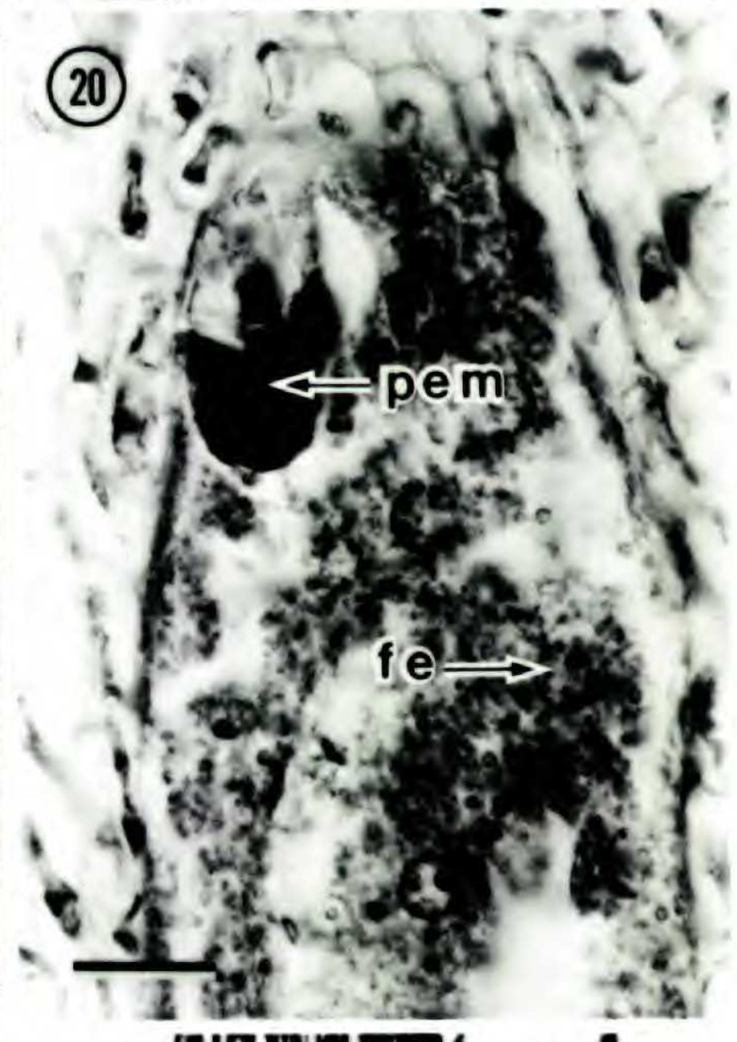
mature ovule at the 8-nucleate embryo sac stage. Of the eight nuclei, two polar nuclei are fused into a single central nucleus, and three antipodal cells are degenerating. Bar = 50 μm.—12, 13. Two serial LSs of a part of the mature ovule showing the egg apparatus and the central nucleus. Bars = 10 μm.—14. Same as Figure 11, but at a lower magnification. Note that the nucellar tissue is persistent and that no cell layer of the integument shows differentiation into an endothelium. Bar = 50 μm. arc, archesporial cell; p, primary parietal cell; s, sporogenous cell; fc, functional megaspore; n, nucleus of the embryo sac; eg, egg cell; ega, egg apparatus; cn, central nucleus; ant, antipodal cell; sy, synergid; nuc, nucellar tissue; in, integument; cp, nucellar cap.

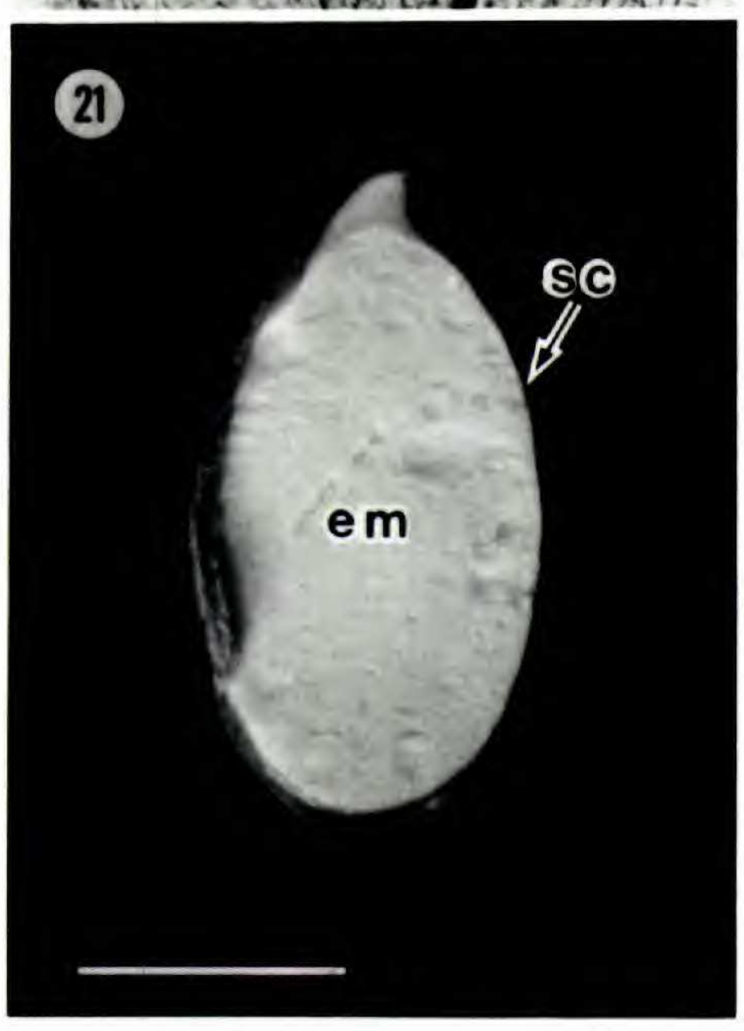


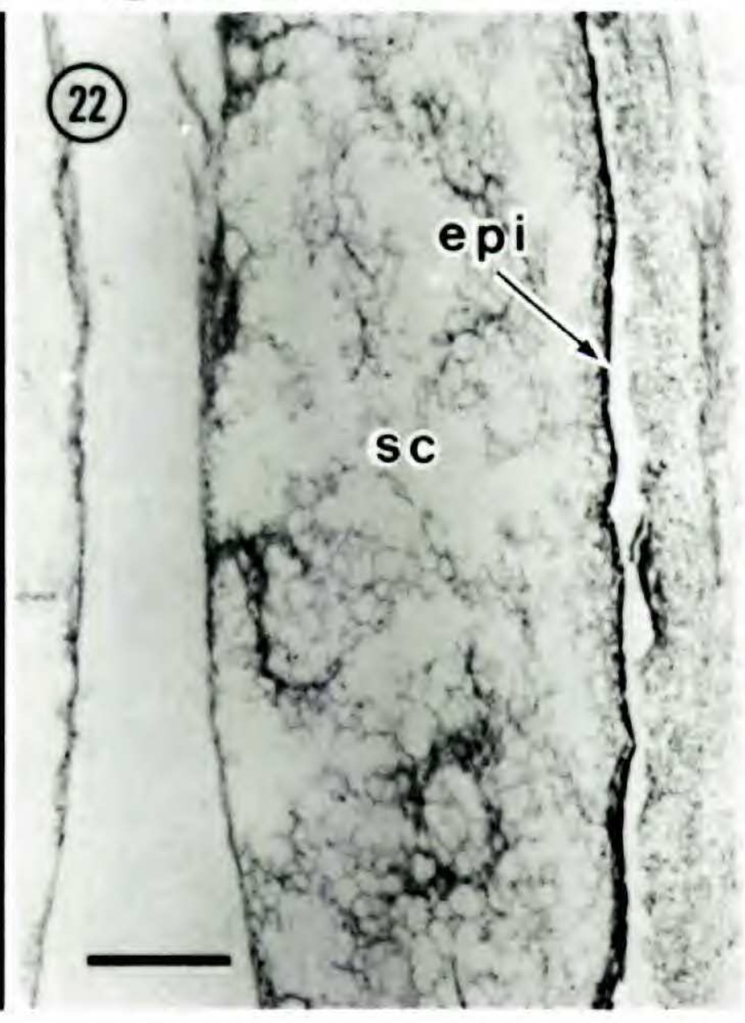












FIGURES 15–23. *Anisophyllea*.—15, 18–20, 22. *A. disticha*.—16, 17, 21, 23. *A.* sp. (*D. W. Thomas 3494*, MO).—15. Longitudinal section (LS) of a young ovule. Note that the ovule is unitegmic. Bar = 50 μm.—16. LS of an unusual mature ovule lacking a micropyle. Bar = 50 μm.—17. Transverse section (TS) of a mature ovule. Bar = 50 μm.—18. LS of a mature ovule tangentially cut through a raphe showing the ramification of a raphe bundle. Bar = 100 μm.—19. TS of a young seed. Note that the thick seed coat contains several vascular bundles at the peripheral part. Bar = 1 mm.—20. LS of a young seed containing a proembryo and free endosperm

functional chalazal megaspore separate from each other: one moves toward the micropylar end, while the other moves toward the chalazal end (Fig. 33). Each nucleus divides successively to form a 4- and an 8-nucleate sac (Fig. 34). Thus the embryo sac formation conforms to the bisporic *Allium* type. The synergids are slightly hooked, and the antipodals are ephemeral, disappearing before fertilization. Two polar nuclei do not fuse with each other until fertilization takes place; they are positioned near the egg apparatus. A mature embryo sac just before fertilization is composed of five nuclei or cells: an egg cell, two synergids, and two polar nuclei.

Embryo sacs characteristically accumulate an abundance of starch grains (Fig. 35). The starch grains, which begin to accumulate from the 2-nucleate embryo sac stage, are most abundant in the 8-nucleate embryo sac stage but disappear after fertilization.

During megasporogenesis and megagametogenesis, the nucellar tissue does not show any particular differentiation and persists at least until the earliest fruit stages (Figs. 36, 38). Apical dermal cells of the nucellus do divide periclinally (Fig. 37), and their daughter cells also repeat periclinal divisions, thus forming a nucellar cap four to six cell layers thick above the embryo sac (Fig. 36).

Integument. The ovule is unitegmic (Figs. 31, 36). The growing integument is about four or five cells thick (Figs. 36; see also Fig. 38). No difference in thickness exists between the different parts of the ovule. Therefore, except for the raphe, the cross section of ovule is nearly circular (Fig. 38). The integument is not vascularized. Neither secondary multiplication of the integument nor differentiation of the innermost cell layer into a so-called endothelium occur.

The integument elongates beyond the nucellar apex and forms a micropyle (Fig. 36).

Fertilization, endosperm, and embryo. The fruits are always one-seeded. Fertilization is porogamous. After fertilization, the fruit elongates remarkably (Fig. 39). Endosperm formation is of the Nuclear type (Fig. 40). In the early stages, free endosperm nuclei are located around the proembryo (Fig. 40) and at the peripheral region of the embryo sac (Fig. 43). Because of incompleteness of our fruit sample, we could not observe to what degree an amount of endosperm increases later. The mature seeds lack endosperm (Figs. 41, 42). We did not investigate embryogenesis in detail but can state, on the basis of our observations of a few microtome-sectioned proembryos, that it proceeds normally (Fig. 40).

Within the mature fruit of *Combretocarpus,* the embryo is elongate and nearly circular in cross section (Figs. 41, 42). The embryo is dicotyledonous with two small cotyledons and a long hypocotyl (Fig. 41).

The hypostase is not differentiated even after fertilization.

Mature seed and seed coat. The mature seed is linear, 9.5–10.4 mm long and 1.2–1.3 mm thick; it contains several vascular bundles in the raphe (Fig. 42), which are derived by ramification from a raphe bundle. These bundles are restricted to the raphe, never entering the integument or testa.

In the young seed, the seed coat is composed of a tanniferous outer epidermis and a multiple inner layer, which degenerates (Fig. 43). Eventually, in the mature seed, the seed coat comprises only the outer epidermis, which is formed of pigmented, cuboid cells (Fig. 44).

POGA PIERRE

Anther and microspores. The anther is tetrasporangiate. The wall prior to maturation comprises five cell layers: an epidermis, endothecium, two middle layers, and a tapetum (Fig. 45). Wall formation conforms to the Basic type. During maturation, cells of the epidermis as well as of the endothecium enlarge, while the middle layers degenerate (Fig. 46). The tapetum is glandular, and its cells become 2-nucleate (Fig. 46). The two nuclei in a tapetal cell are not fused with each other. The mature anther wall is composed of the persistent epidermis and the endothecium. The epidermis is tanniferous, and the endothecium develops fibrous thickenings (Fig. 47). The anther dehisces by longitudinal slits. By the time of dehiscence, the connective tissue between two

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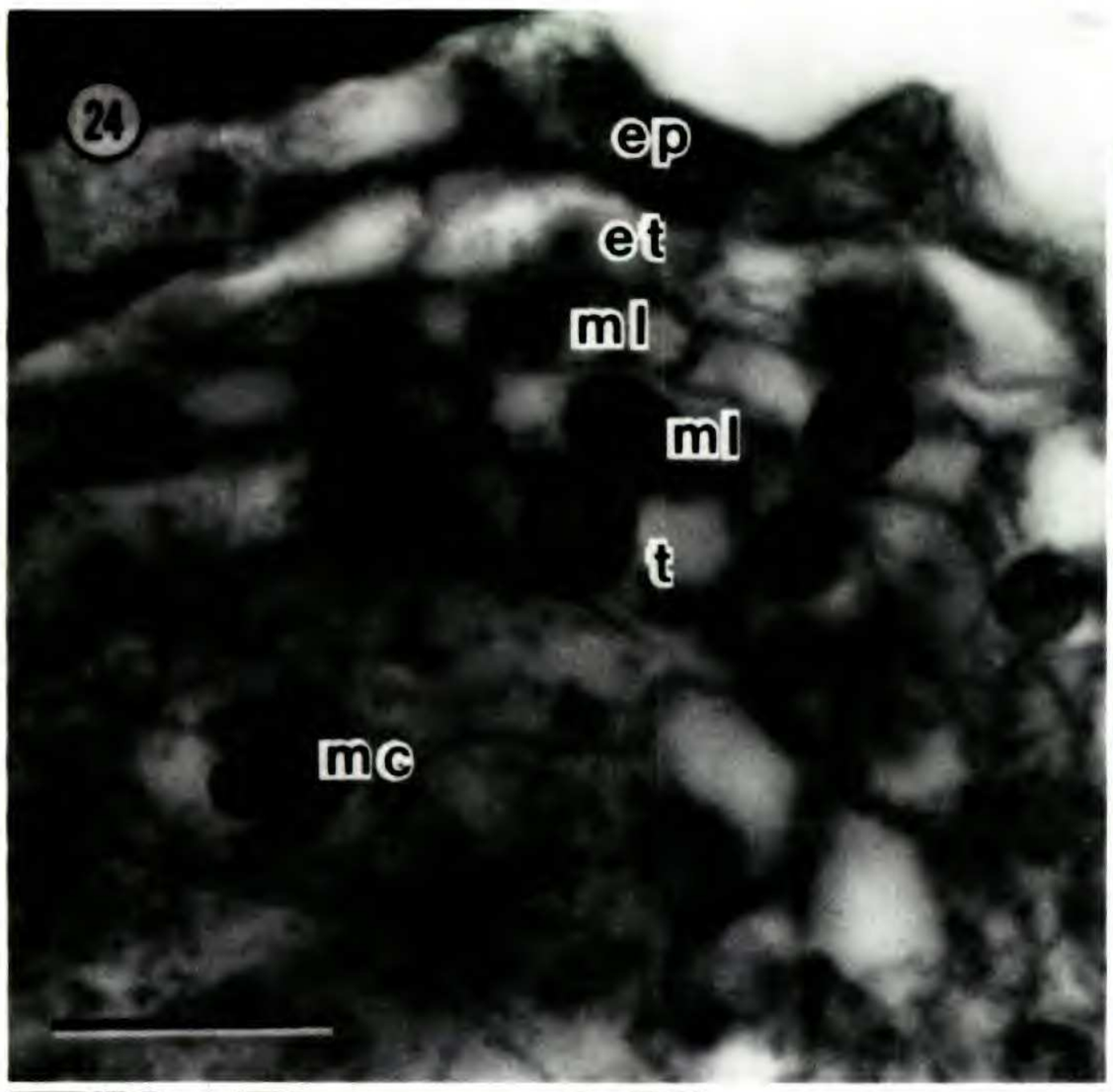
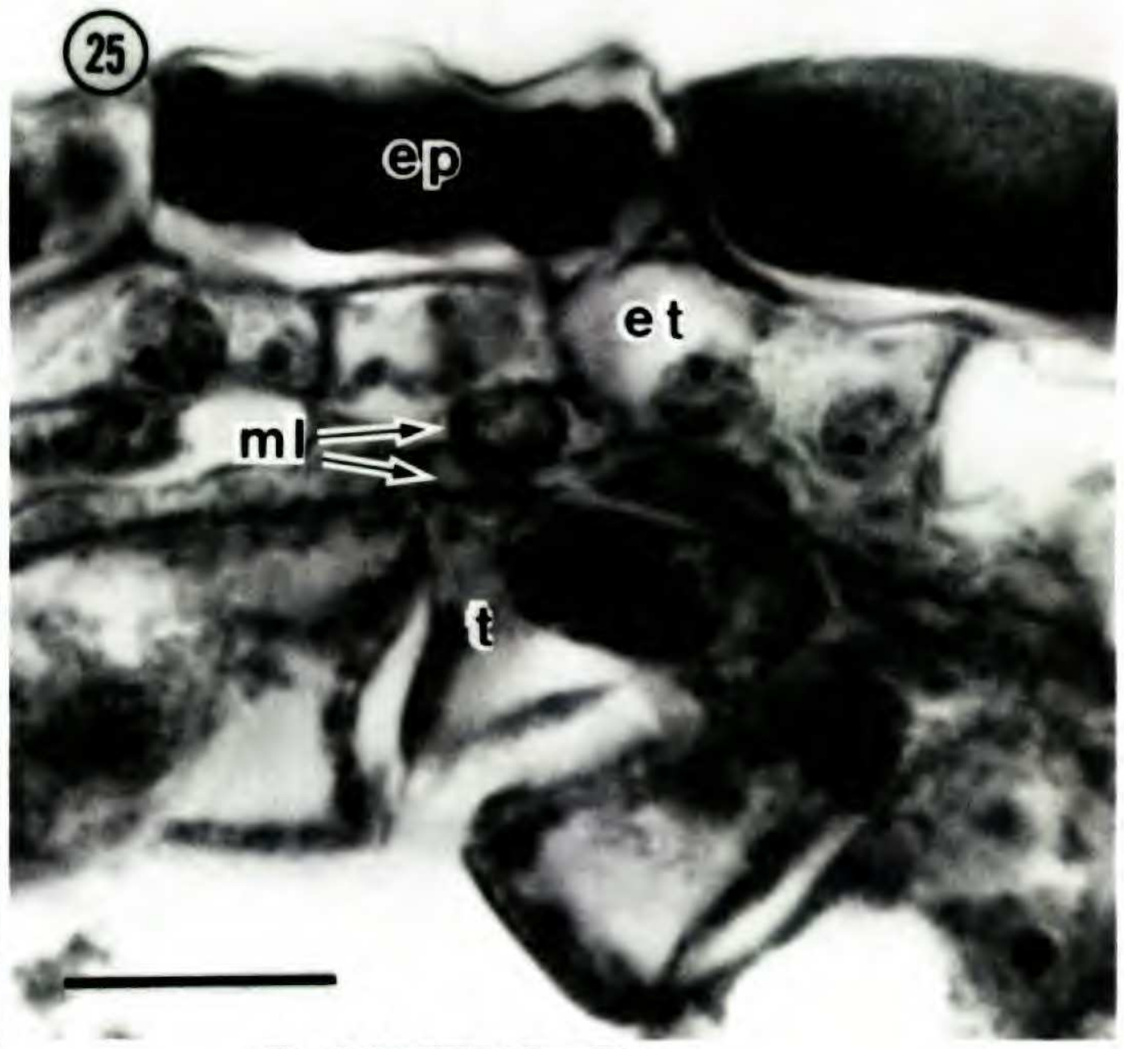
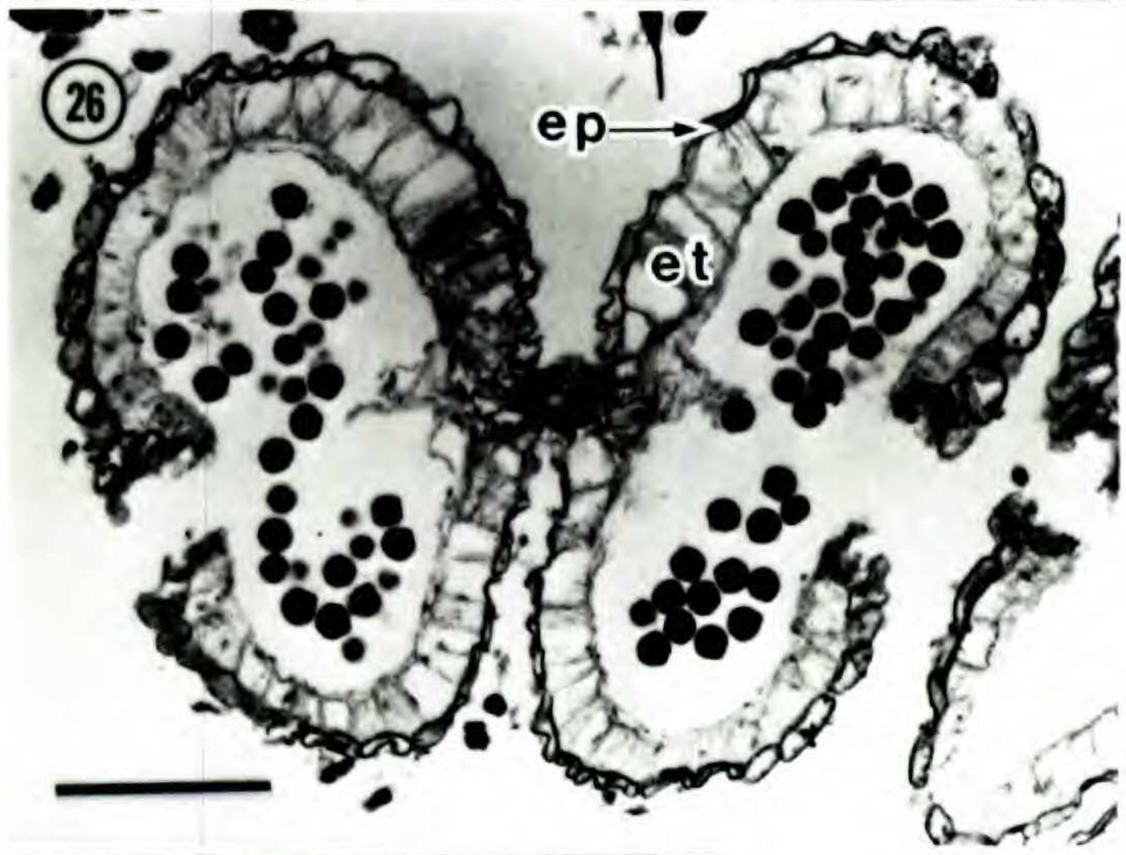
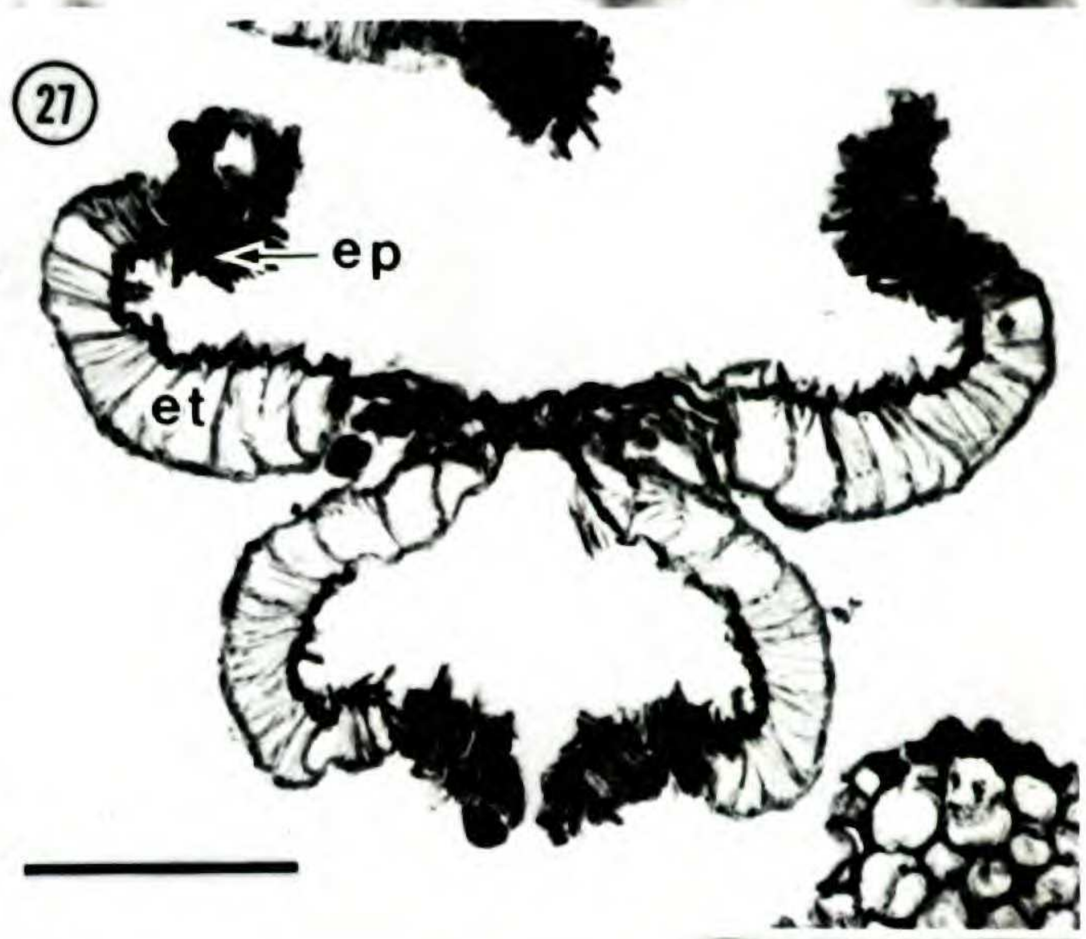
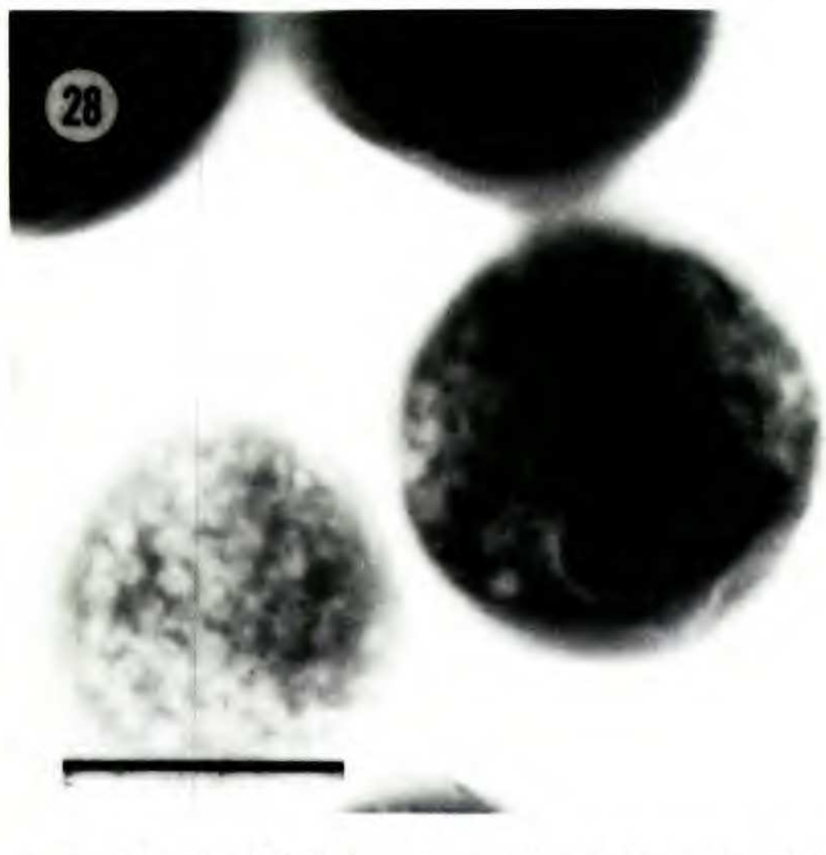
nuclei. Bar = 20 μm.—21. Longitudinal hand-section of a mature seed. Note that the mature seed is exalbuminous. Bar = 5 mm.—22. LS of a young seed showing a thick seed coat. Bar = 200 μm.—23. LS of a mature seed coat that is formed by both the multiple inner layer and the conspicuous outer epidermis. Bar = 40 μm. in, integument; nuc, nucellar tissue; rb, raphe bundle; b, vascular bundle; pem, proembryo; fe, free endosperm nucleus; em, embryo; sc, seed coat; epi, epidermis of seed coat; inl, multiple inner layer.

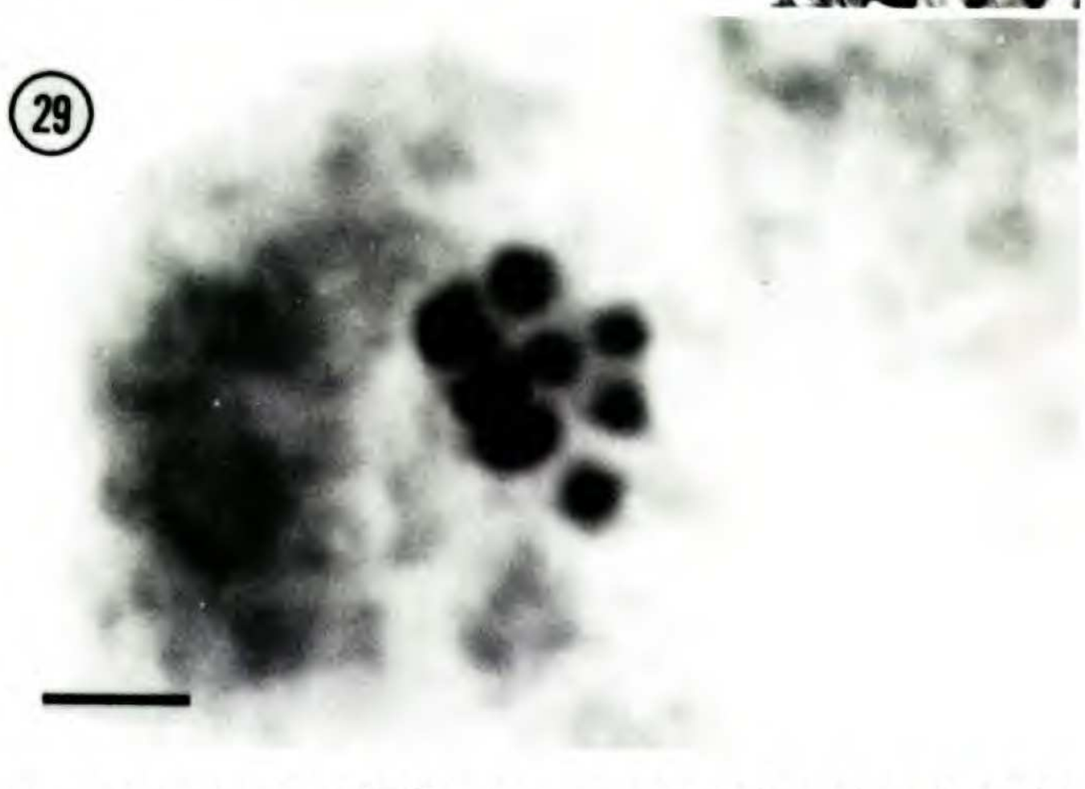












FIGURES 24–29. *Combretocarpus rotundatus.*—24. Transverse section (TS) of a young anther showing the five cell-layered wall structure. Bar = 10 μm.—25. TS of an older anther with degenerating middle layers. Bar = 10 μm.—26. TS of an anther at the time of dehiscence. Its wall comprises the fibrous endothecium and the epidermis. Bar = 50 μm.—27. TS of an older anther than that shown in Figure 26. The anther wall is remarkably reflexed. Bar = 50 μm.—28. Two-celled mature pollen at the time of shedding. Bar = 10 μm.—29. Chromosomes of pollen mother cell at metaphase I. $n = 8$. Bar = 2 μm. ep, epidermis; et, endothecium; ml, middle layer; t, tapetum; mc, microspore mother cell.

microsporangia of each theca degenerates completely.

Meiosis in the microspore mother cell is accompanied by simultaneous cytokinesis. The shape of the resultant tetrads, on the basis of the examination of 50 selected tetrads are "usually" (86%) tetrahedral, "occasionally" (12%) decussate, and "rarely" (2%) isobilateral. The pollen grains are 2-celled at the time of shedding (Fig. 48).

Chromosomes. Using serially sectioned pollen mother cells that were fixed at the later prophase of meiosis I, we observed the chromosomes of *Poga* for the first time and determined the chromosome number $n = 7$ (Figs. 49–51).

Nucellus and integuments. Although female flowers were not available, we confirmed by using mature ovules and very young fruits that the nucellar tissue enclosing the embryo sac persists at least until the early stages of fruit development (Figs. 52, 54). No hypostase is differentiated even after fertilization.

The ovule is bitegmic, i.e., possessing the outer and the inner integument (Fig. 52). The outer integument is originally about four or five cells thick, and the inner integument two cells thick (Fig. 53). The cells of the outer epidermis of the outer integument, which later become those of the outermost layer of the exotesta, are conspicuously enlarged into cuboid cells. The raphe bundle ramifies and vascularizes the outer integument. In a cross section of a young fruit, six to eight vascular bundles in addition to several raphe bundles are observed in the testa (Fig. 54).

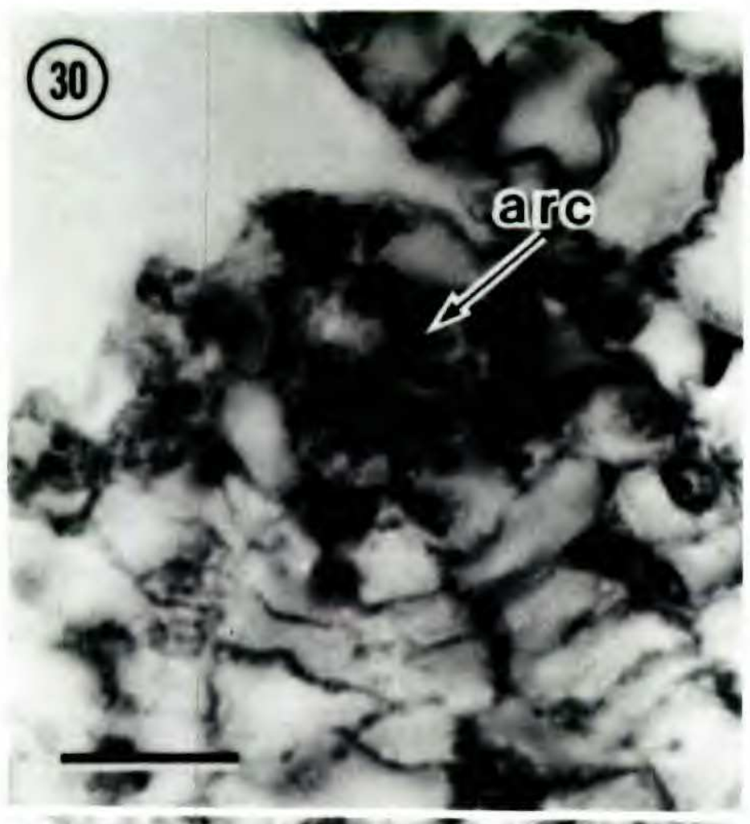
The micropyle is formed by both integuments (Fig. 52).

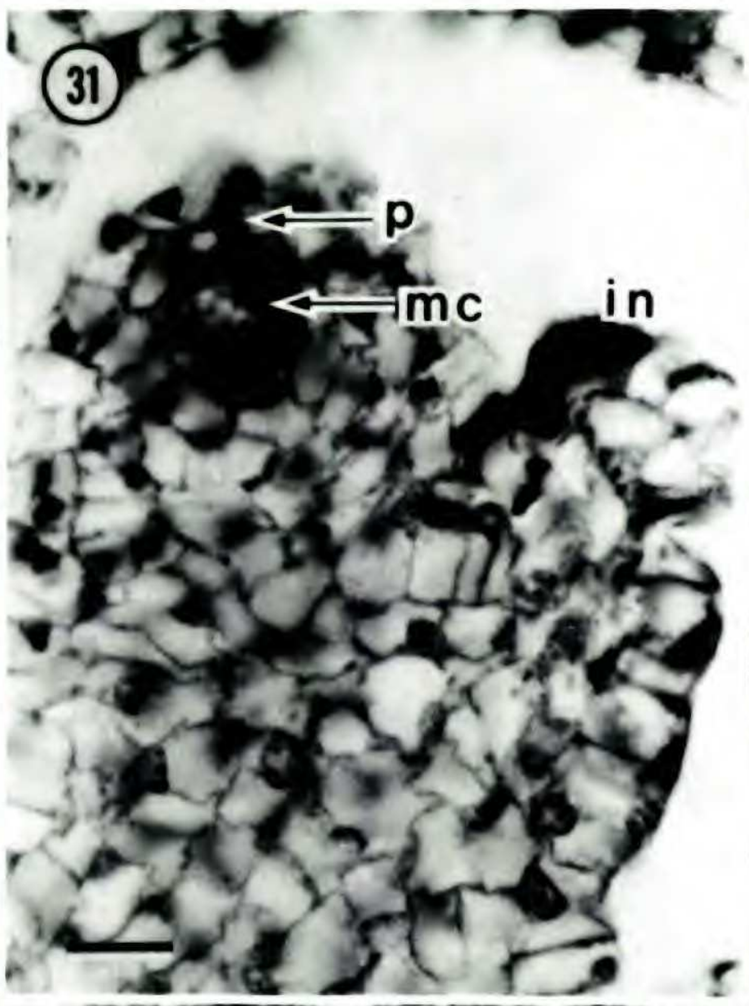
Endosperm and embryo. We could not observe either the mode of endosperm formation or embryogenesis. But we can say at least that the mature seed completely lacks endosperm (Figs. 55, 56) as Vaughan (1970) described, and that the embryo does not have cotyledons. Concerning the cotyledons, Vaughan (1970) mentioned that they are fused. However, judging from the resemblance in exomorphology of the embryo with *Anisophyllea,* it seems that *Poga* also lacks cotyledons from the beginning.

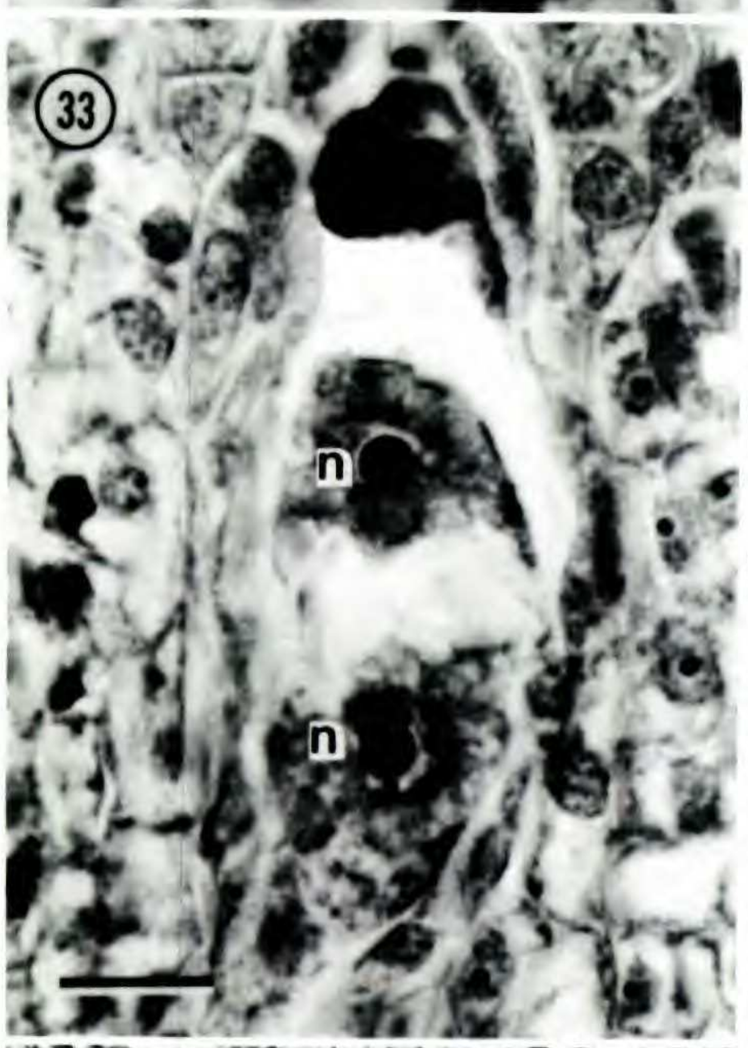
Mature seed and seed coat. The fruits are always one-seeded. The mature seed is 20.0–22.5 mm long and 12.0–13.5 mm thick, is ovoid and slightly suppressed toward the raphe-antiraphe direction, and has a thick, dark brown seed coat (Figs. 55, 56).

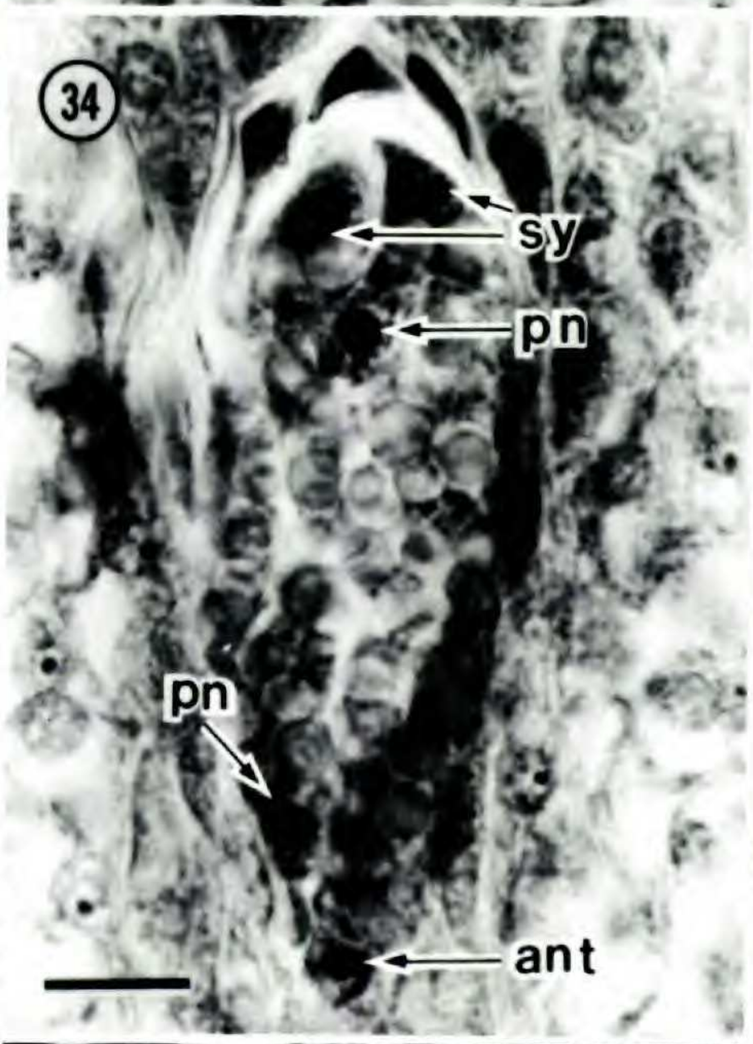
In the young seed, the seed coat is composed only of a thick testa and lacks a tegmen. It seems that, during the process of seed development, the inner integument or tegmen is crushed, while the outer integument or testa increases in thickness by secondary multiplication. Within the young testa, a differentiation into a multiple outer layer and a multiple inner layer can be observed (Fig. 57). The multiple outer layer is about 8 cells thick and has cells that are more or less enlarged. In contrast, the multiple inner layer is 7–10 cells thick, with the cells stretched tangentially (Fig. 57). The structure of the mature seed coat basically does not differ from that of the young seed coat. In the mature seed coat, however, the walls of the constituent cells are thickened, and the multiple inner layer occupies nearly one-third of the whole thickness of the testa, with the multiple outer layer occupying the remaining two-thirds (Fig. 58). Because our microtome sections of the seed coat were not very good, we could not examine the details of cell structure. Vaughan (1970), however, gave a drawing of the anatomical structure of the testa, which consists of an inner layer that is about 12 cells thick and an outer layer about six or seven cells thick. Referring to Vaughan (1970), Corner (1976) described the outer epidermis of the multiple outer layer as composed of cuboid cells with slightly thickened, lignified walls, and the other cells of the multiple outer layer as thin-walled.

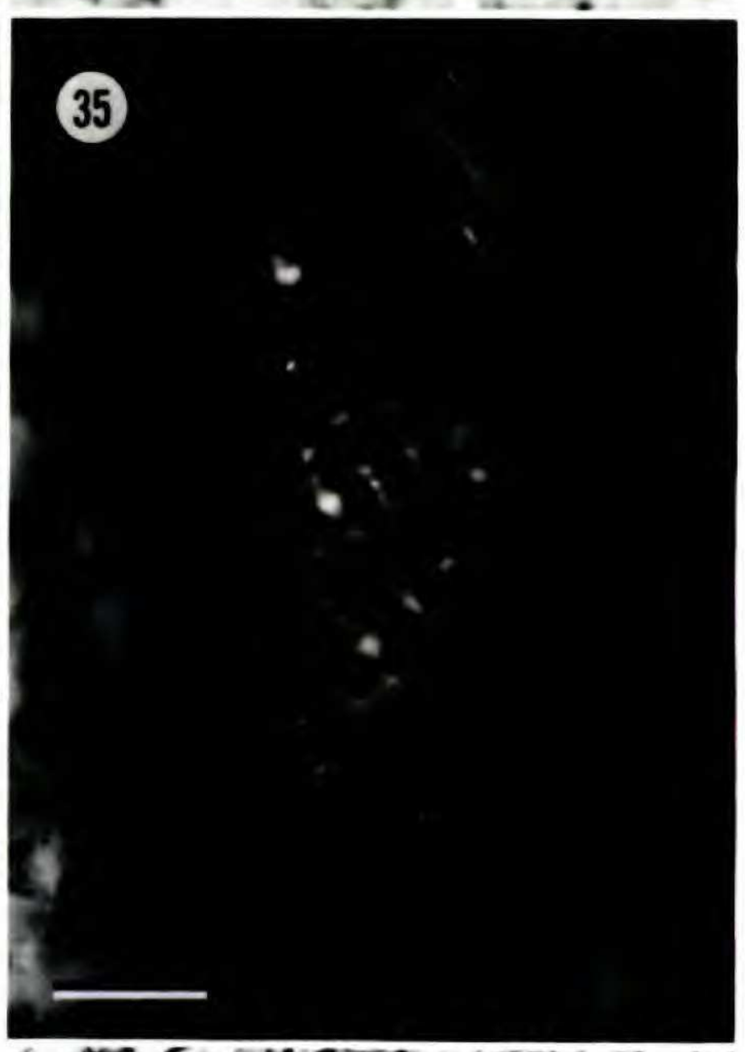
The mature seed coat structure of *Poga,* which is bitegmic, seems comparable with that of *Anisophyllea,* which is unitegmic. In both genera, the mature seed coat contains a similar (probably identical) multiple inner layer, which is characteristically composed of tangentially stretched cells with thick walls. The only evident difference between the mature seed coat structure of *Poga* and that of *Anisophyllea* lies in thickness of the outer layer, i.e., about six or seven cells thick in *Poga* (see Vaughan, 1970) and one cell thick (outer epidermis only) in *Anisophyllea.* In other words, the seed coat of *Anisophyllea,* like that of *Poga,* may also be constructed principally of the "testa" (or "outer integument"), which of course is not differentiated in the single integument of *Anisophyllea.* Therefore the seed of unitegmic *Anisophyllea* and even of unitegmic *Combretocarpus* (with a mature seed coat consisting only of the outer epidermis) may be regarded as testal, and the seed of bitegmic *Poga* can also be defined in this way.

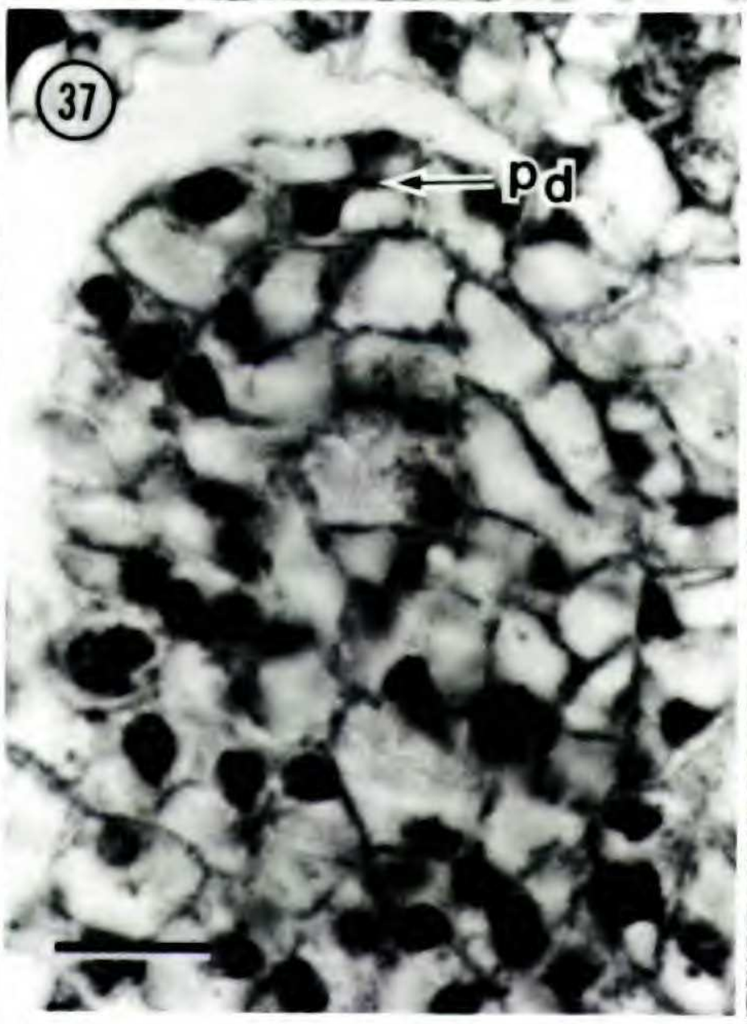


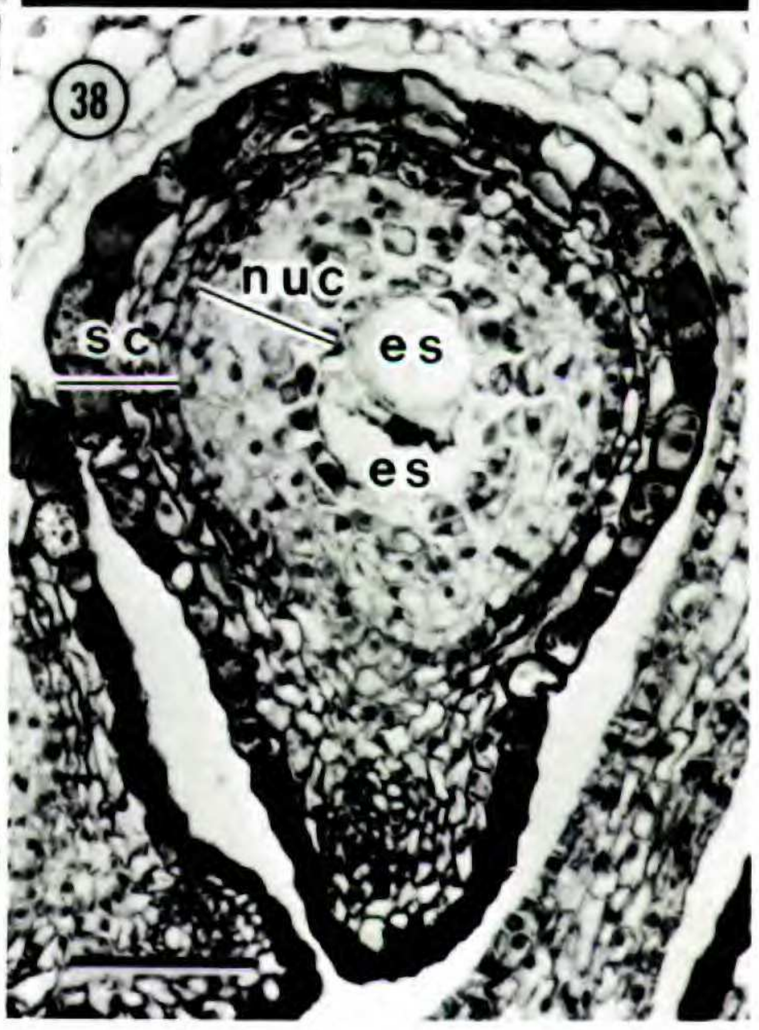












FIGURES 30–38. *Combretocarpus rotundatus*.—30. Longitudinal section (LS) of an ovule with the 1-celled archesporium. Bar = 10 μm.—31. LS of a young ovule with the primary parietal cell and the megaspore mother cell. Note that the ovule has only a single integument. Bar = 10 μm.—32. LS of a young ovule with the megaspore dyad. Note that each megaspore has two nuclei. Bar = 10 μm.—3. LS of an ovule at the 2-nucleate embryo sac stage. Bar = 10 μm.—34. LS of a nearly mature ovule at the 8-nucleate embryo sac stage. Bar = 10 μm.—35.

POLYGONANTHUS DUCKE

Anther and microspores. The anther is basically tetrasporangiate. The microsporogenous tissue, however, is occasionally transversely divided by a septum composed of tapetal cells (Fig. 59). Although we could not determine the modes of anther wall formation, the wall prior to maturation comprises five cell layers: an epidermis, endothecium, two middle layers, and a tapetum. During maturation, the middle layers degenerate, while the cells of both the epidermis and the endothecium become enlarged (Fig. 59). The tapetum is glandular, and its cells become 2-nucleate before degeneration (Fig. 60). The two nuclei in a tapetal cell do not fuse with each other. Eventually the mature anther wall is composed of a persistent epidermis, whose cells are somewhat collapsed in places, and a fibrous endothecium (Fig. 61). The connective tissue between two microsporangia of each theca degenerates completely before the anthers dehisce.

Meiosis in the microspore mother cells is accompanied by simultaneous cytokinesis. The pollen grains are 2-celled at the time of shedding (Fig. 62).

Chromosomes. Using serially sectioned microspore mother cells that were fixed at the late prophase of meiosis I, the chromosomes of *Polygonanthus* were observed for the first time. Throughout, at examination and reconfirmation in many cells, we determined the chromosome number of *P. amazonicus* as $n = 7$ (Figs. 63–65).

Megagametophyte and nucellus. Although our observations are fragmentary, we were able to observe some aspects of the process of megasporogenesis and megagametogenesis in *Polygonanthus.* The ovule is anatropous and crassinucellate. At least one parietal cell is cut off above the megaspore mother cell (Figs. 66, 67). Although the mode of embryo sac formation was not determined, the 2-nucleate embryo sac of *Polygonanthus amazonicus* differs in aspect from that of *Combretocarpus rotundatus* (which develops a bisporic *Allium* type embryo sac) (Fig. 68; compare with Fig. 33). The accumulation of starch grains in the embryo sac, which is characteristic of *Combretocarpus,* does not occur in *Polygonanthus amazonicus.* The antipodal cells are probably ephemeral, because they are absent in the organized mature embryo sacs (Fig. 69).

The nucellar tissue enclosing the embryo sac is persistent until at least the stage of fertilization (Fig. 69). Periclinal divisions occur in the apical dermal cells of the nucellus. Therefore the nucellar cap is probably formed by derivatives of the apical dermal cells.

Integuments. The ovule is bitegmic, i.e., it has both an outer and an inner integument (Fig. 66). The outer integument is initially about five cells thick, but later becomes seven to nine or more cells thick because of secondary multiplication (Fig. 66). The inner integument, in contrast, is two cells thick (Fig. 67). In the later stages, the inner integument becomes very much less conspicuous, while the outer integument increases in thickness (Fig. 69). Although we could not observe any stages of the development of the seed coat, it seems very unlikely that the inner integument or tegmen contributes to its structure when mature. The raphe bundle ramifies throughout the outer integument, which is therefore vascularized. In cross section, seven or eight bundles in addition to several raphe bundles are observed (Fig. 70).

The micropyle is formed by both integuments (Fig. 69).

DISCUSSION

Our own results on the embryology and chromosome numbers of Anisophylleaceae, together with some data on ovule and seed morphology published earlier (Karsten, 1891; Vaughan, 1970; Geh & Keng, 1974), are presented in Table 2. On this basis, we summarize the embryological features of Anisophylleaceae as follows.

Anther tetrasporangiate, but occasionally polysporangiate because of insertion of tapetal septa (*Polygonanthus*); anther wall with five cell

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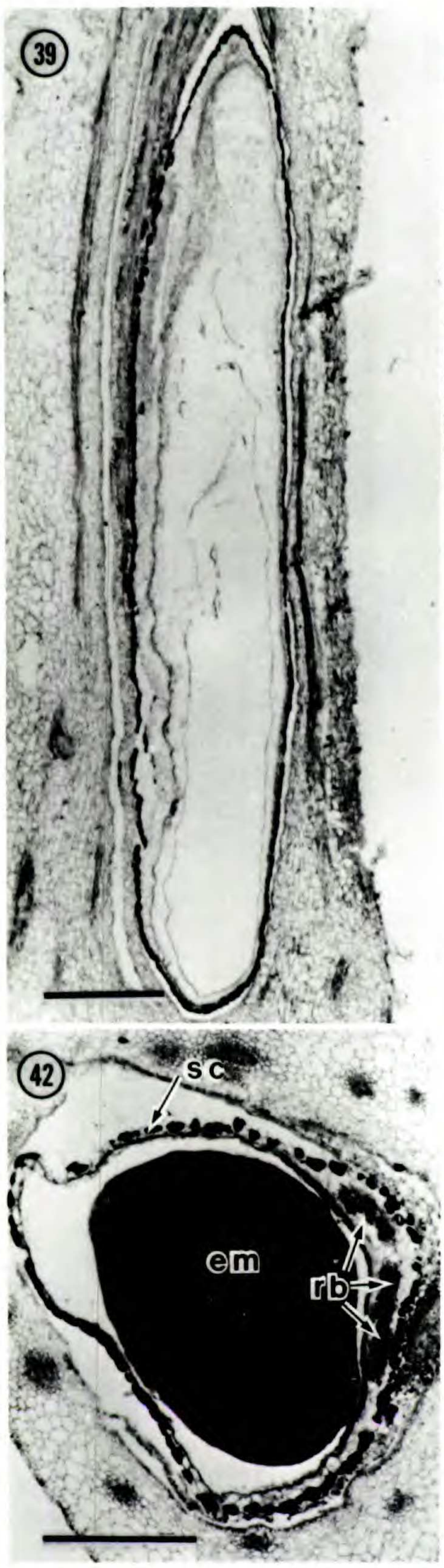
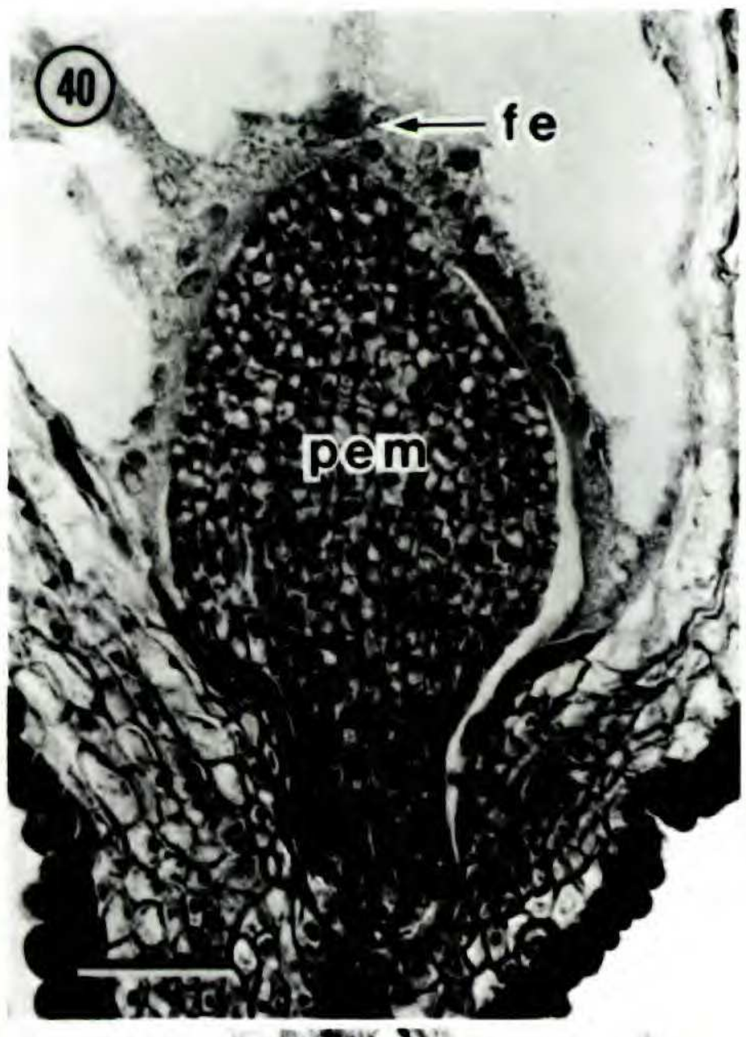
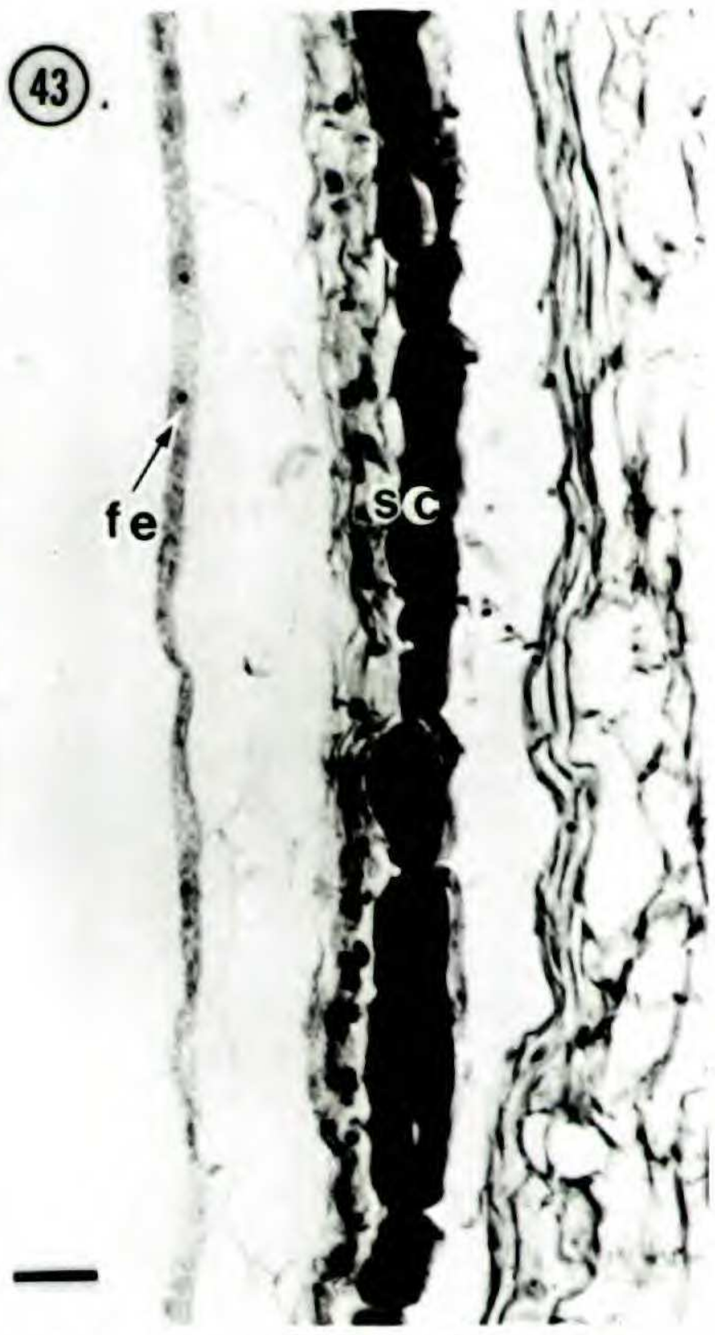
Polarized view of the same as that shown in Figure 34, showing a conspicuous accumulation of starch grains in the embryo sac. Bar = 10 μm.—36. LS of a mature ovule with an organized embryo sac. Note that the nucellar tissue is persistent. Bar = 50 μm.—37. LS of a young ovule nearly at the megaspore dyad stage showing periclinal divisions occurring in apical epidermal cells of the nucellus. Bar = 10 μm.—38. Transverse section of a young seed with twin embryo sacs. Bar = 50 μm. arc, archesporial cell; p, primary parietal cell; mc, megaspore mother cell; in, integument; n, nucleus of the embryo sac; sy, synergid; pn, polar nucleus; ant, antipodal cell; nuc, nucellar tissue; pd, periclinal cell division; sc, seed coat; es, embryo sac.

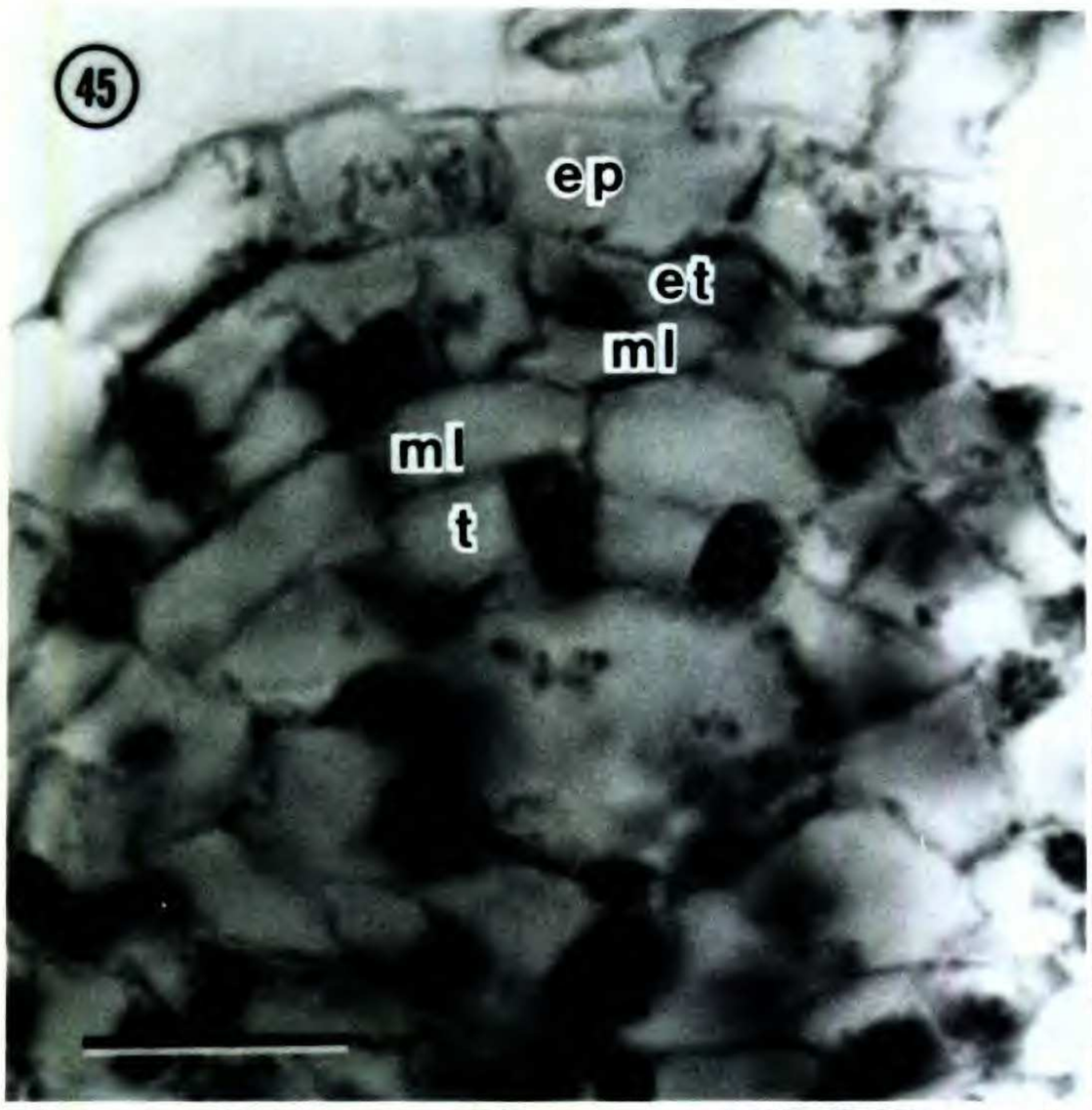


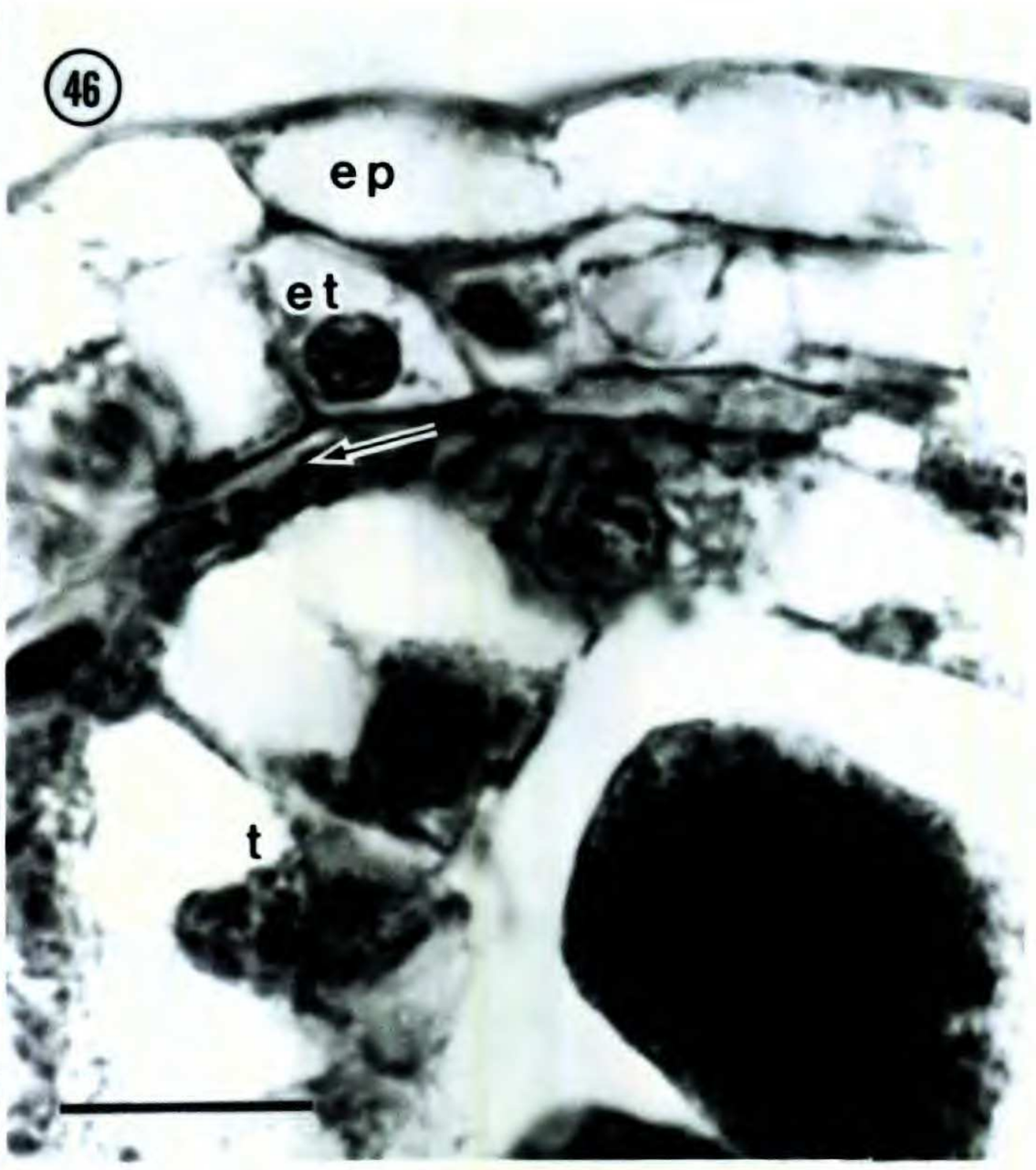


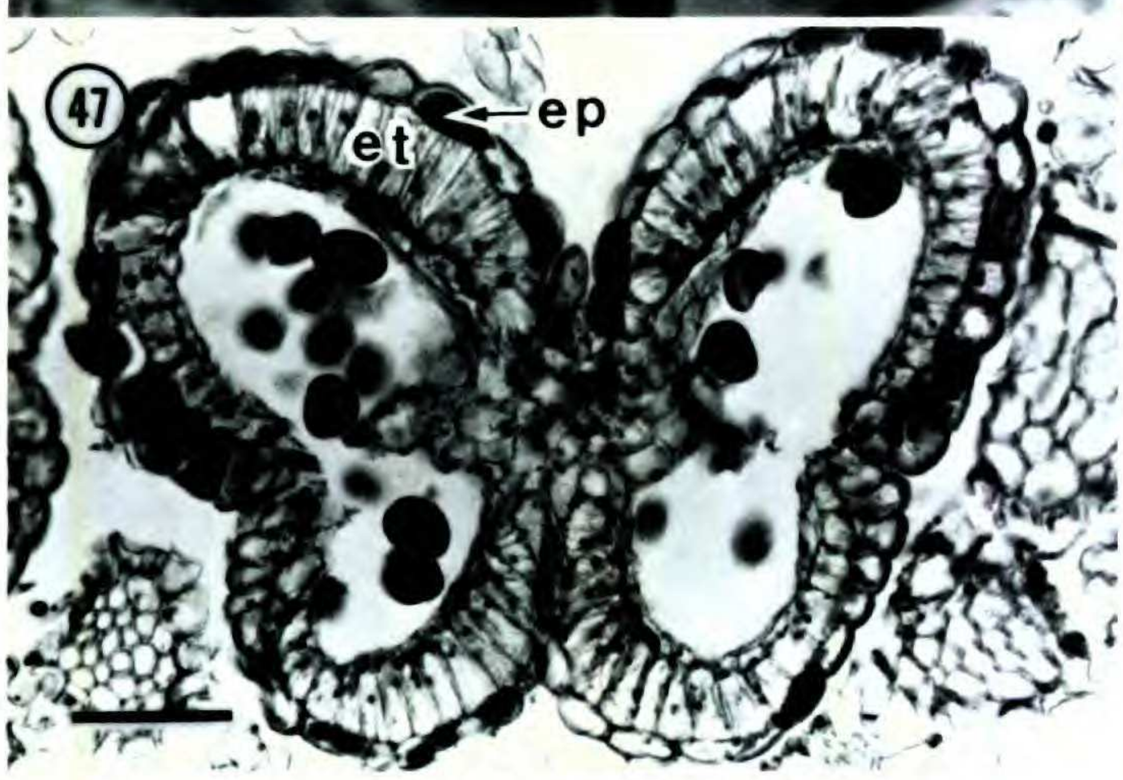


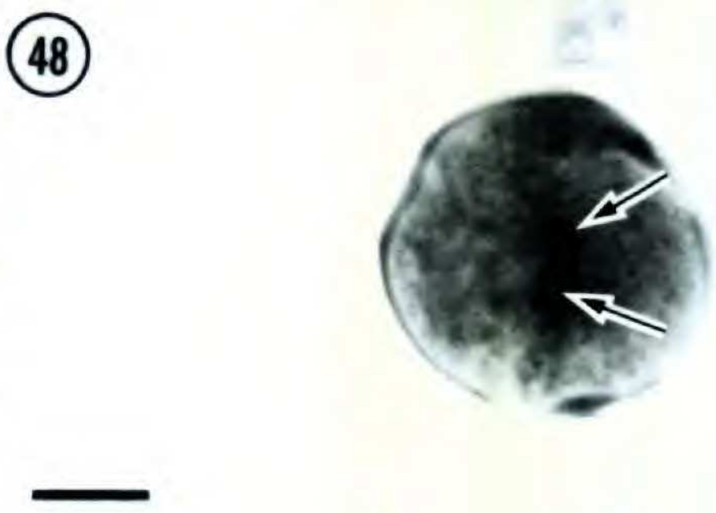
FIGURES 39–44. *Combretocarpus rotundatus*.—39. Longitudinal section (LS) of a remarkably elongated young fruit. Bar = 500 μm.—40. LS of a proembryo with free endosperm nuclei surrounding it. Bar = 50 μm.—41. LS of a mature seed with a cotyledonous embryo. Note that the mature seed is exalbuminous. Bar = 1 mm.—42. Transverse section of a mature seed with several vascular bundles at the raphe. Bar = 500 μm.—43. LS of a young seed coat. Bar = 10 μm.—44. LS of a mature seed coat. Bar = 10 μm. pem, proembryo; fe, free endosperm nucellus; cot, cotyledon; em, embryo; sc, seed coat; rb, raphe bundle.

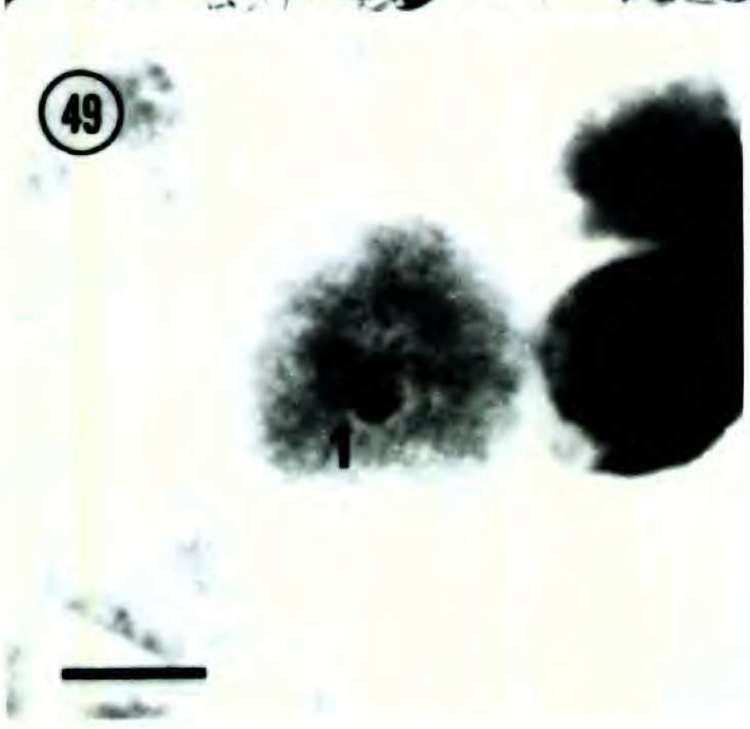
layers, its formation of the Basic type; anther epidermis persistent, consisting of more or less collapsed cells; endothecium persistent and developing fibrous thickenings; middle layers ephemeral; tapetum glandular, its cells 2-nucleate; the two nuclei in each tapetal cell eventually fused in *Anisophyllea* and *Combretocarpus*, but not in *Poga* and *Polygonanthus*.

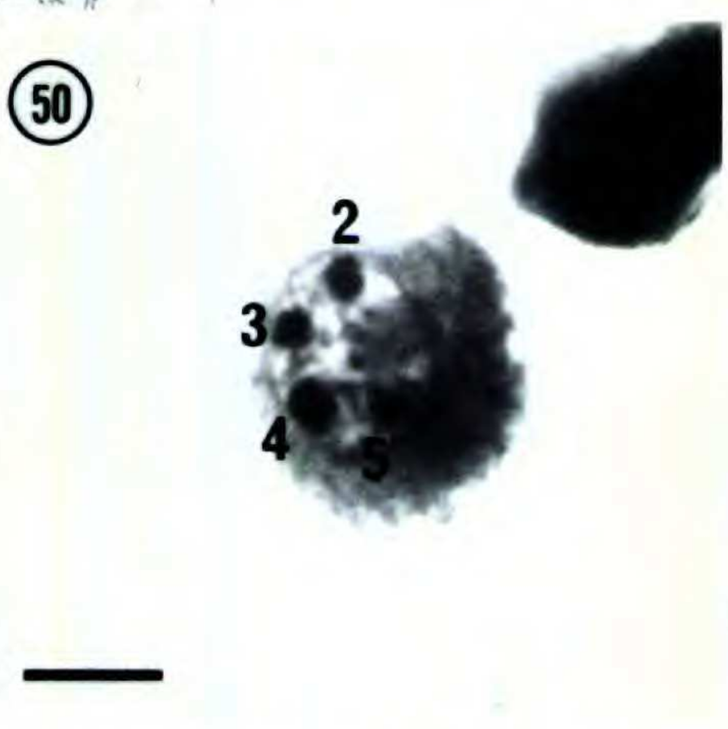


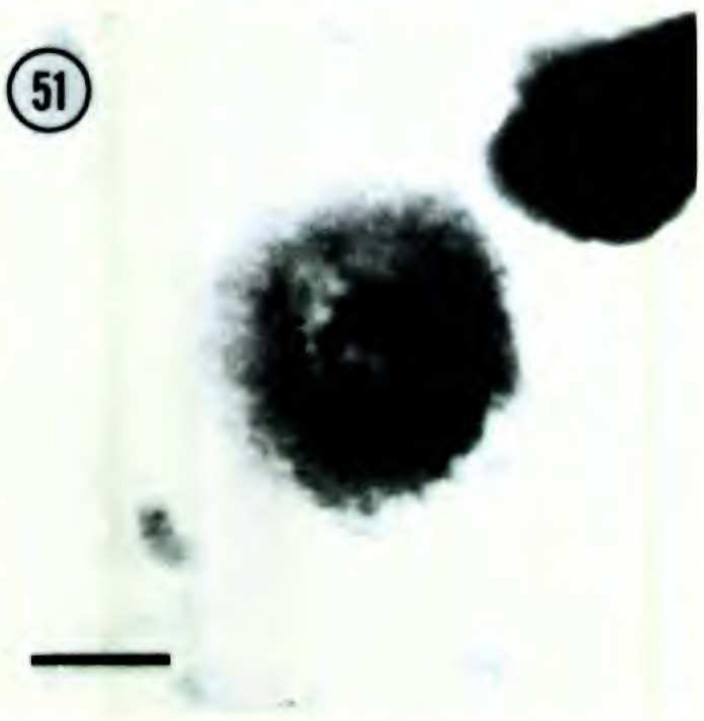










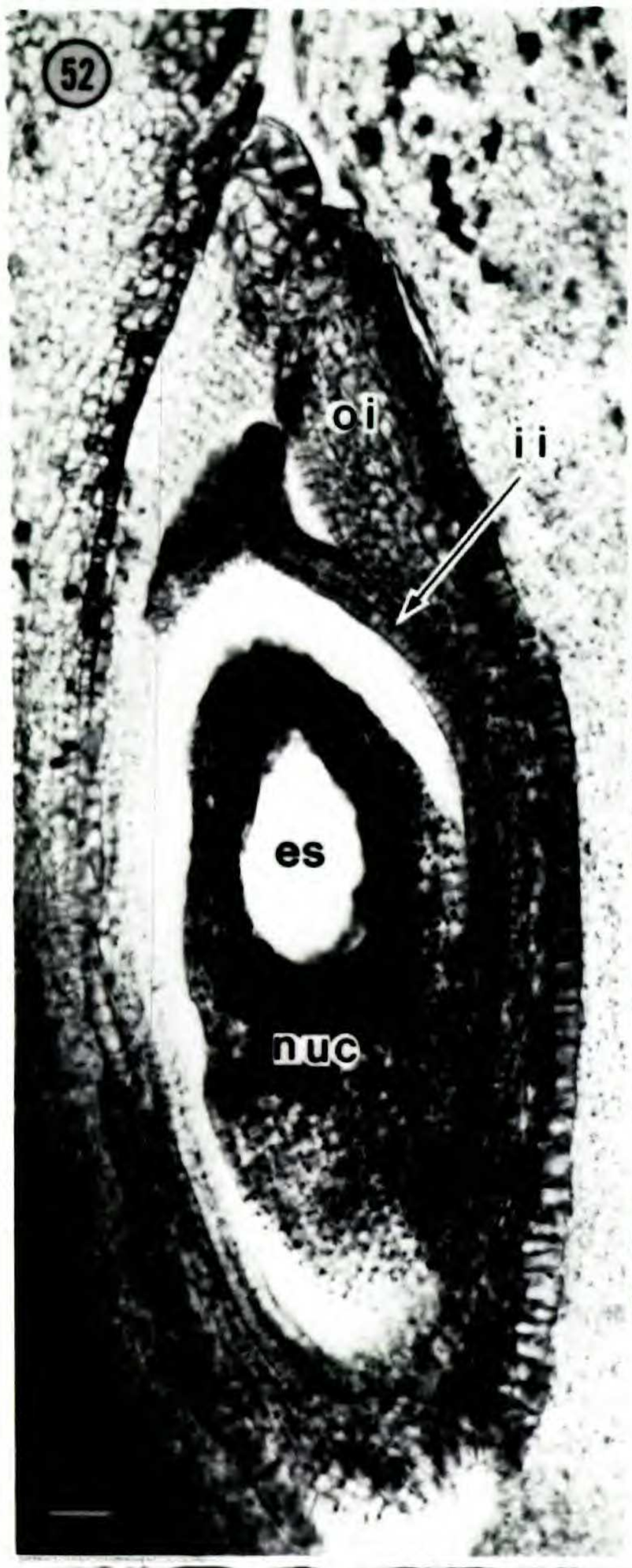


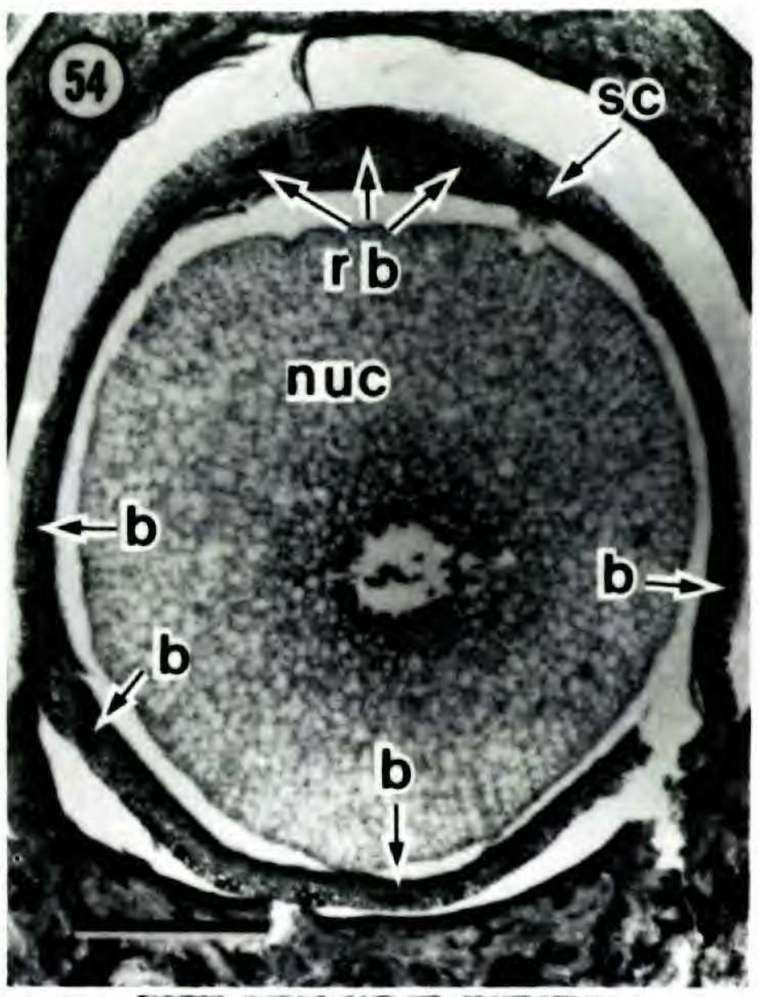
FIGURES 45–51. *Poga oleosa*.—45. Transverse section (TS) of a young anther showing the wall structure with five cell layers. Bar = 10 μm.—46. TS of an older anther with degenerating middle layers (arrow). Bar = 10 μm.—47. TS of a nearly mature anther. Its wall consists of the fibrous endothecium and the epidermis. Bar = 100 μm.—48. Two-celled mature pollen at the time of shedding. Arrows indicate nuclei of the two cells. Bar = 10 μm.—49–51. Three serial sections of pollen mother cell at the late prophase I showing chromosomes of n = 7. Seven chromosomes are numbered 1 to 7. Bars = 10 μm. ep, epidermis; et, endothecium; ml, middle layer; t, tapetum.

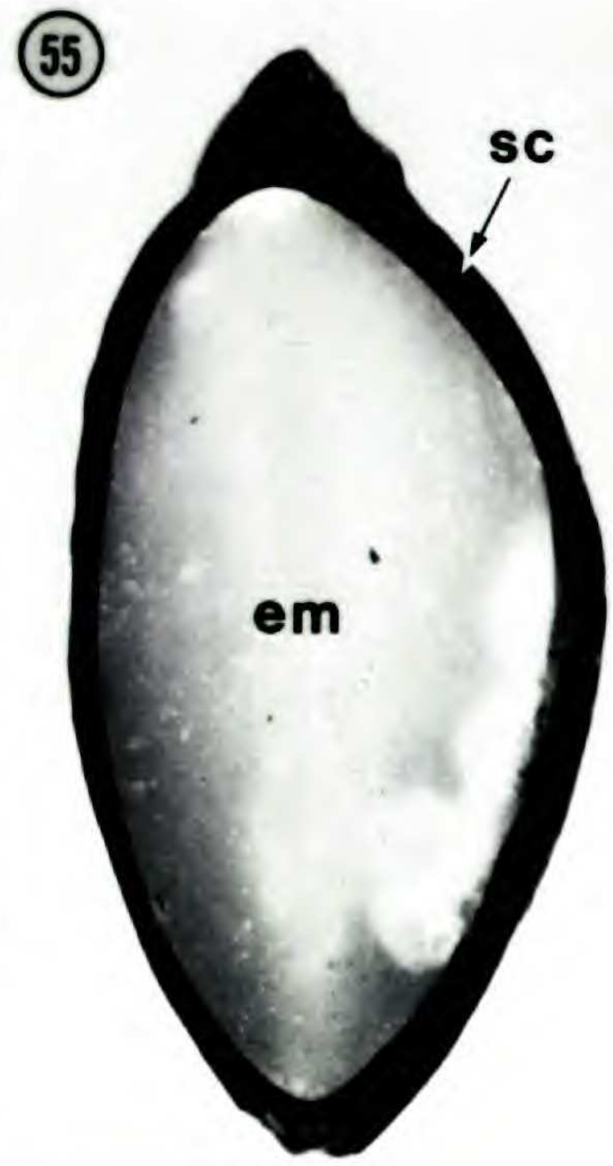
Cytokinesis in the microspore mother cells simultaneous; microspore tetrads tetrahedral, decussate, or isobilateral; pollen grains 2-celled when shed.

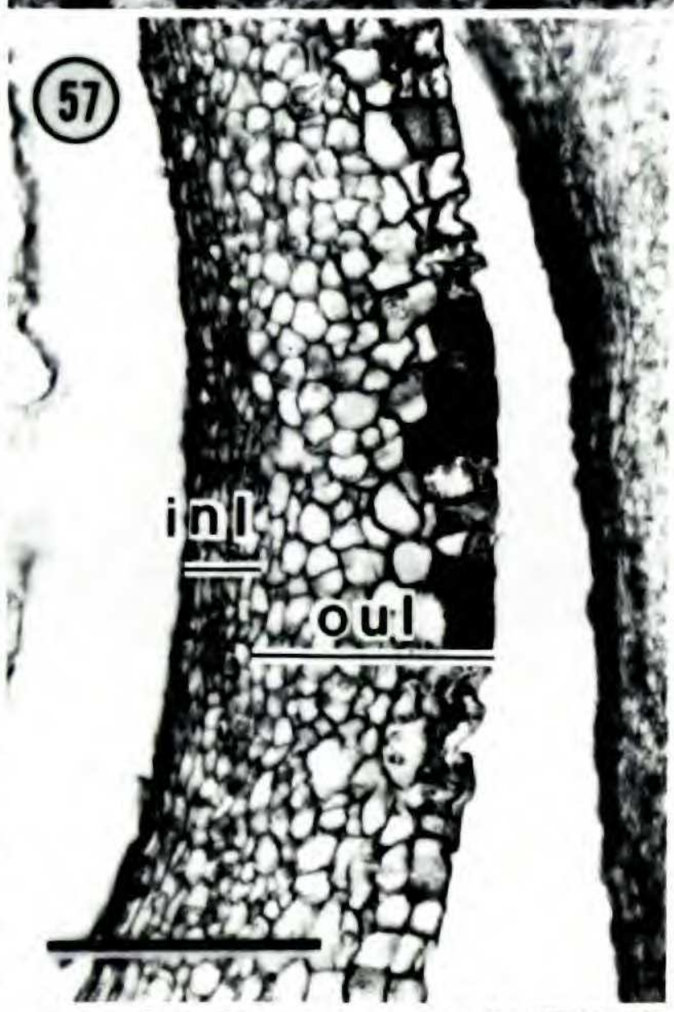
Gametic chromosome number $n = 8$ in *Combretocarpus*, $n = 7$ in *Anisophyllea*, *Poga*, and *Polygonanthus*.

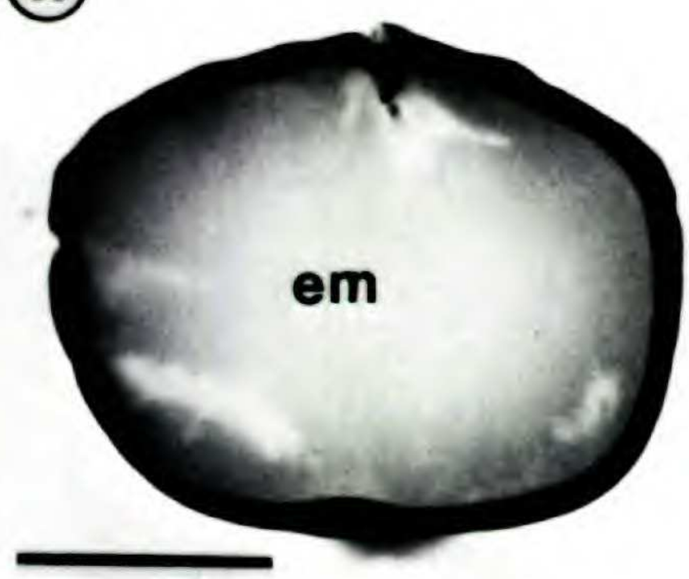
Ovule anatropous and crassinucellate; arche-

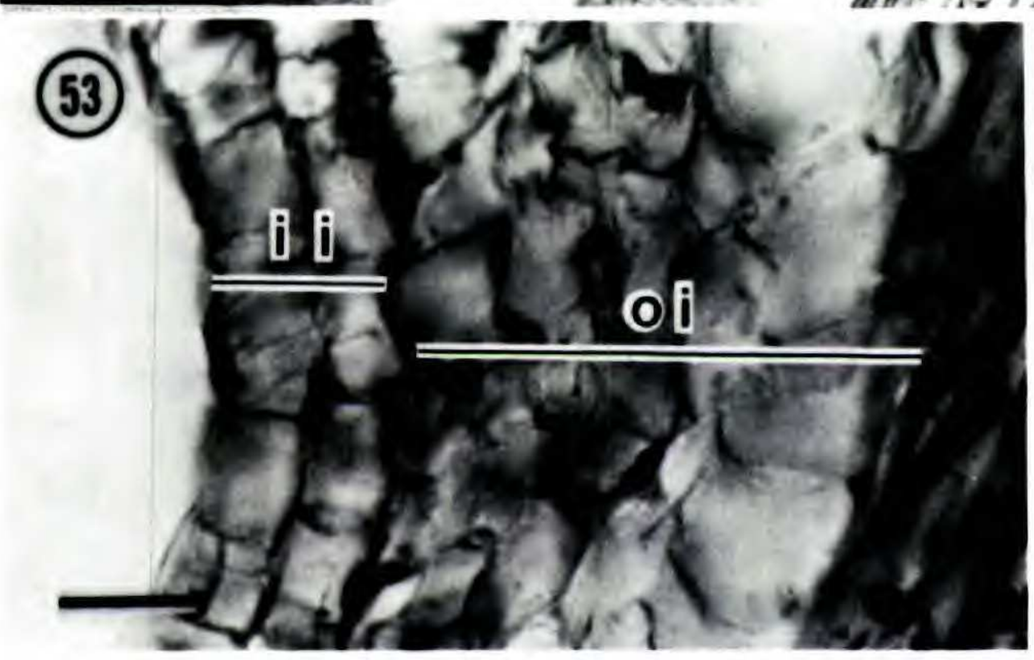


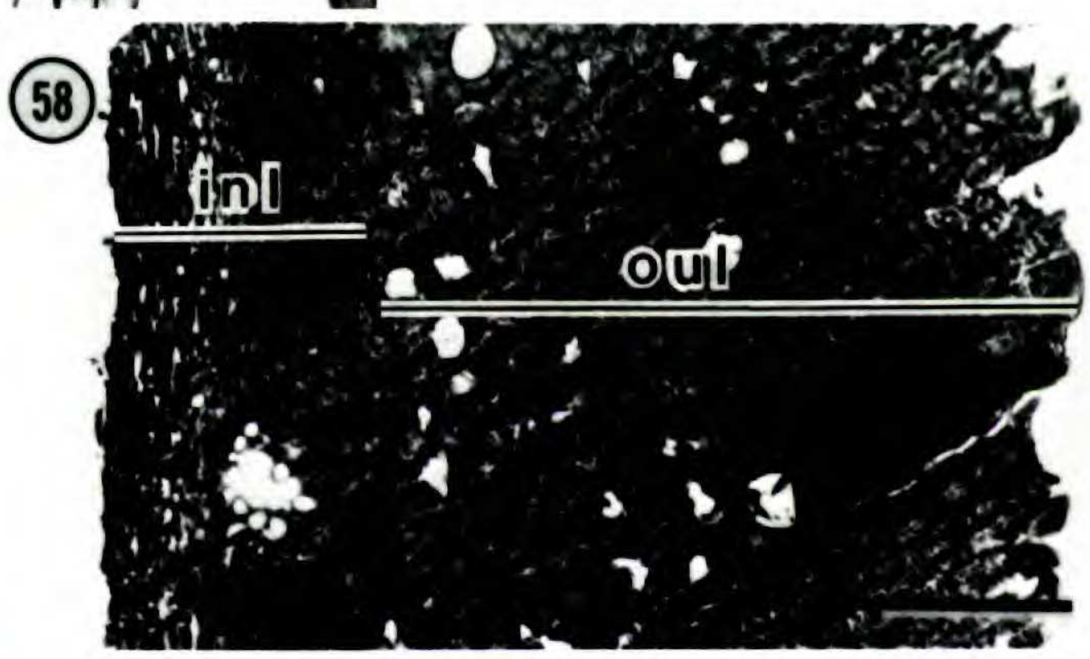




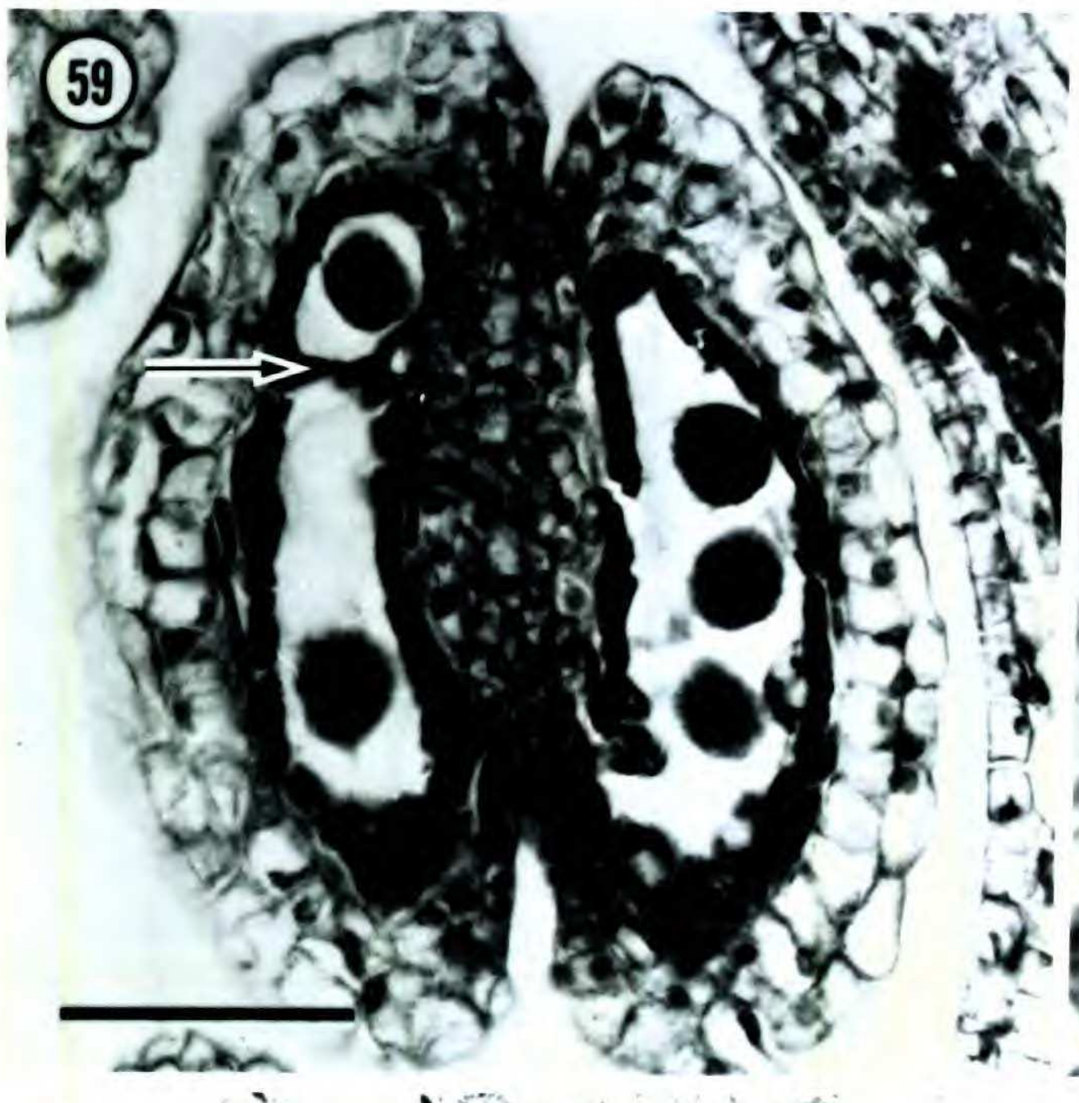


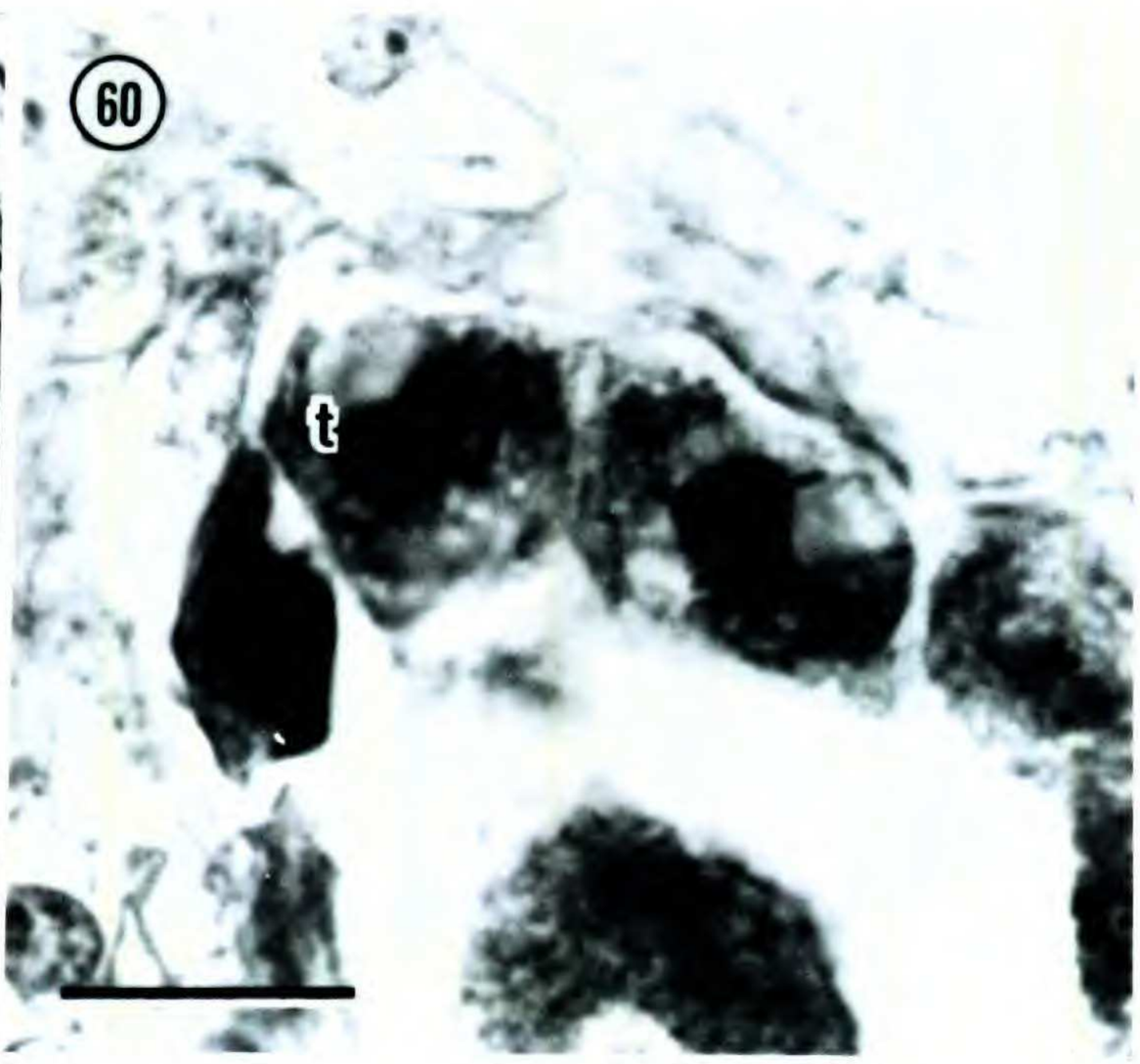


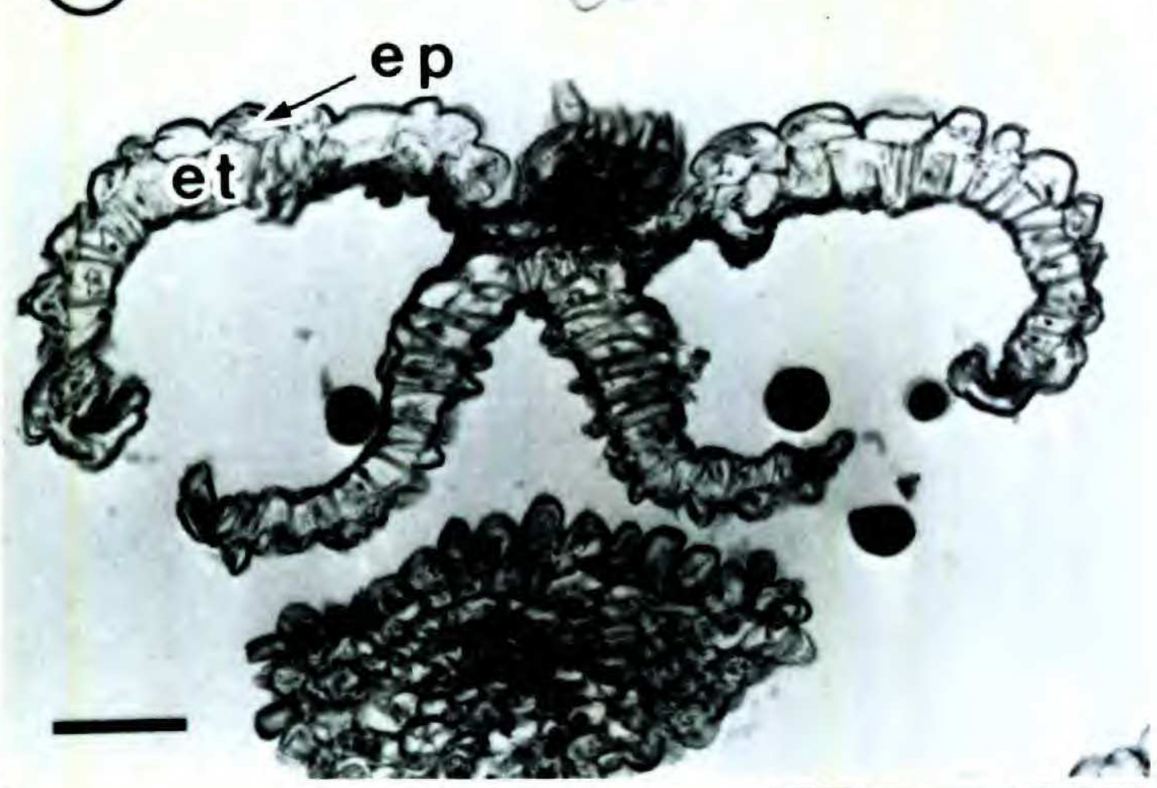


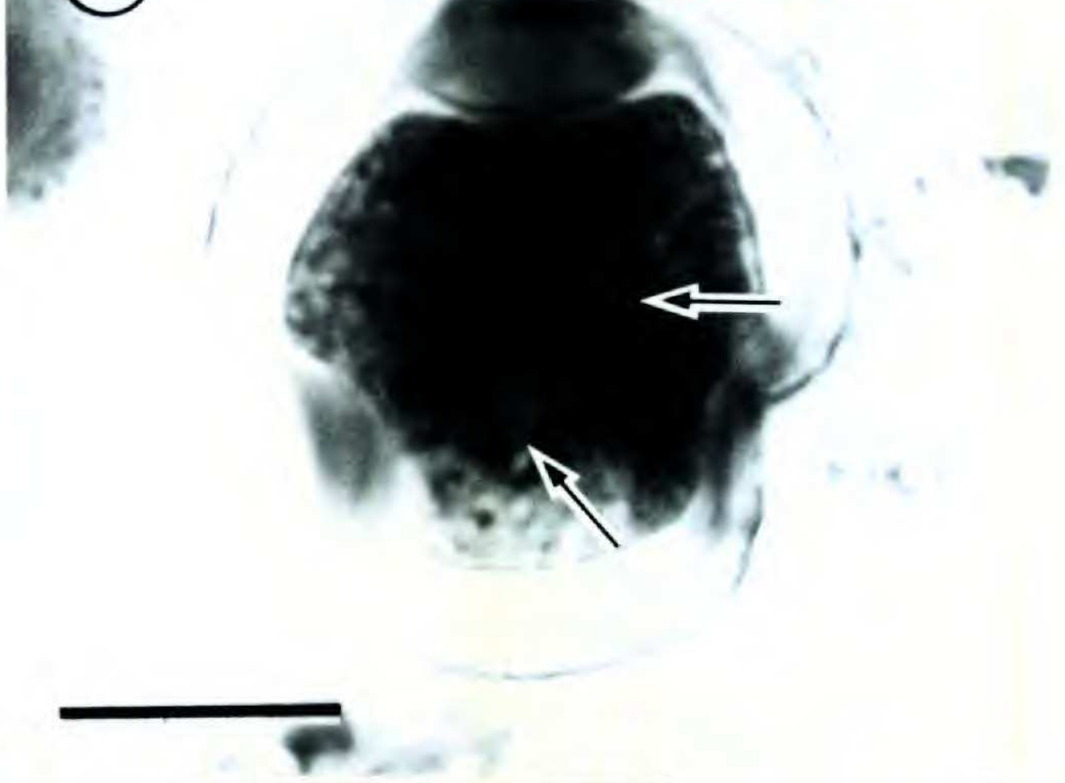


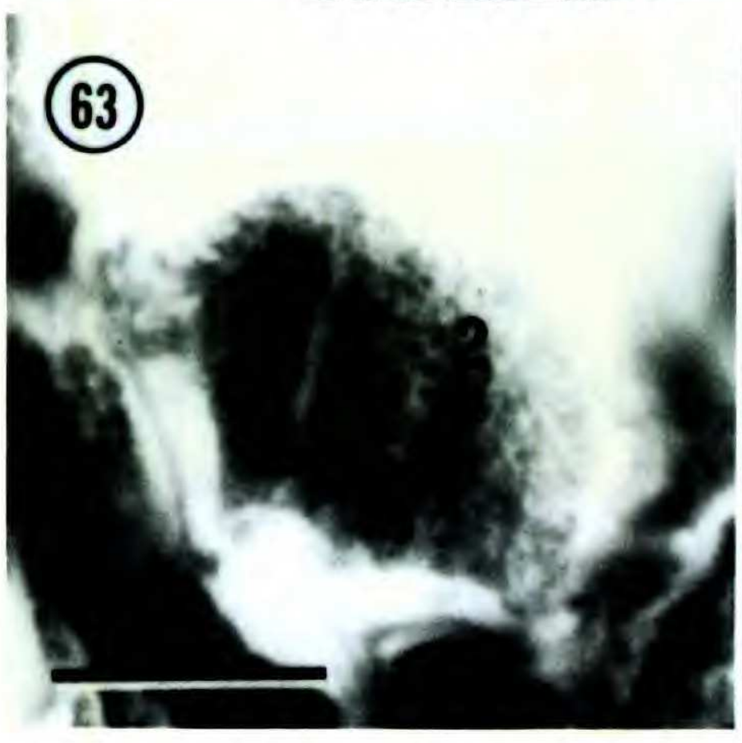
FIGURES 52–58. *Poga oleosa*.—52. Longitudinal section (LS) of a mature ovule. Note that the ovule is bitegmic and has a persistent nucellar tissue. Bar = 100 μm.—53. Transverse section (TS) of a mature ovule showing the inner integument, with two cell layers, and the outer integument, with four cell layers. Bar = 10 μm.—54. TS of a young seed showing the vascularized seed coat. Bar = 500 μm.—55. Longitudinal hand-section of the mature seed. Note that the mature seed is exalbuminous. Bar = 5 mm.—56. Transverse hand-section of the mature seed. Bar = 5 mm.—57. TS of a young seed coat. Note that there is no tegmen and that the testa is differentiating into the multiple inner layer and the multiple outer layer. Bar = 100 μm.—58. LS of a mature seed coat. Bar = 100 μm. ii, inner integument; oi, outer integument; es, embryo sac; nuc, nucellar tissue; rb, raphe bundle; b, vascular bundle; sc, seed coat; em, embryo; inl, multiple inner layer; oul, multiple outer layer.

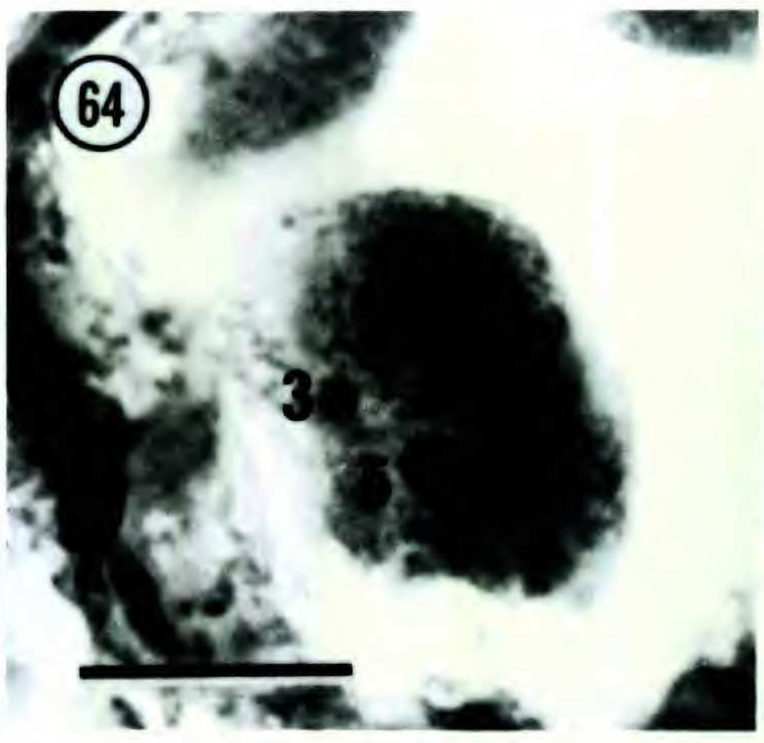


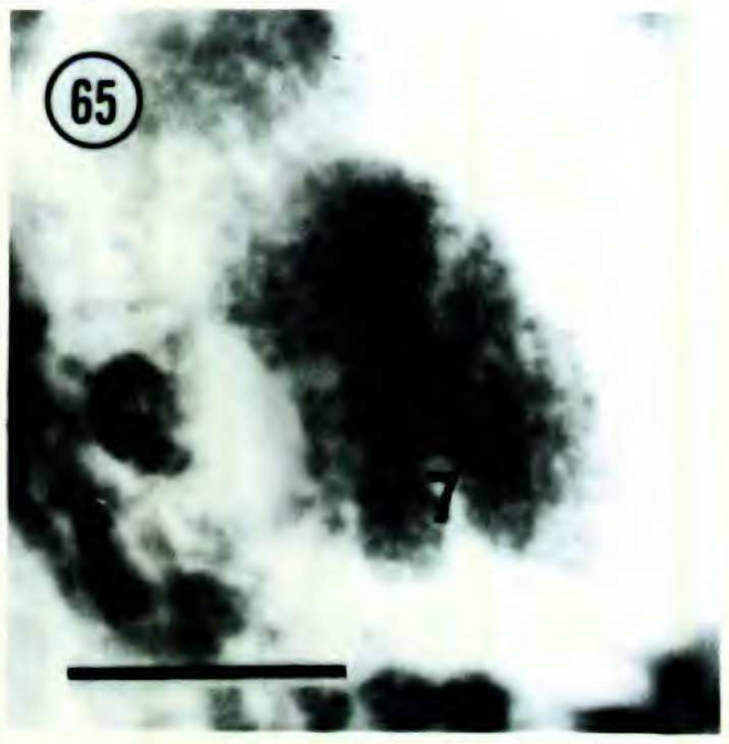




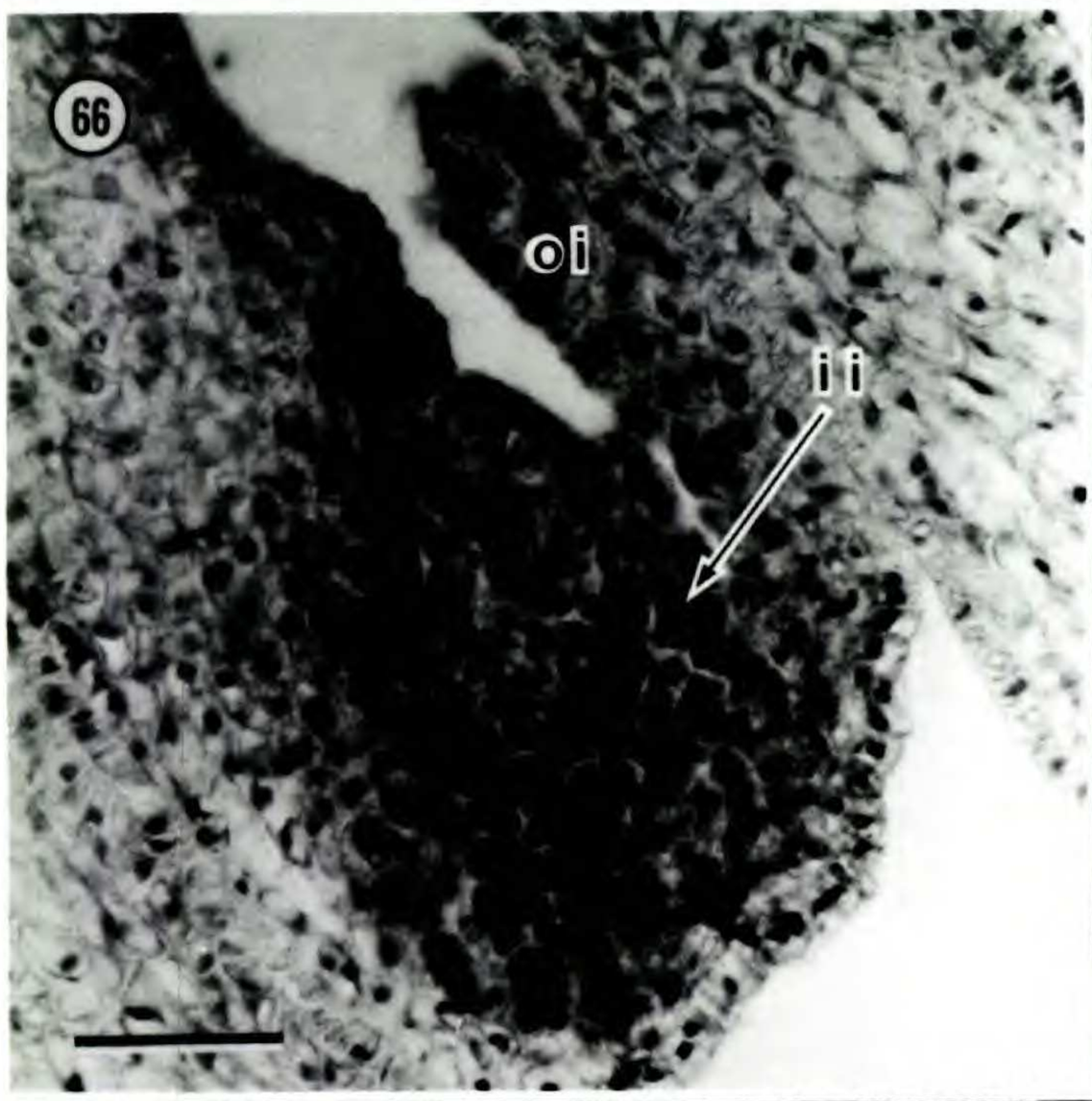


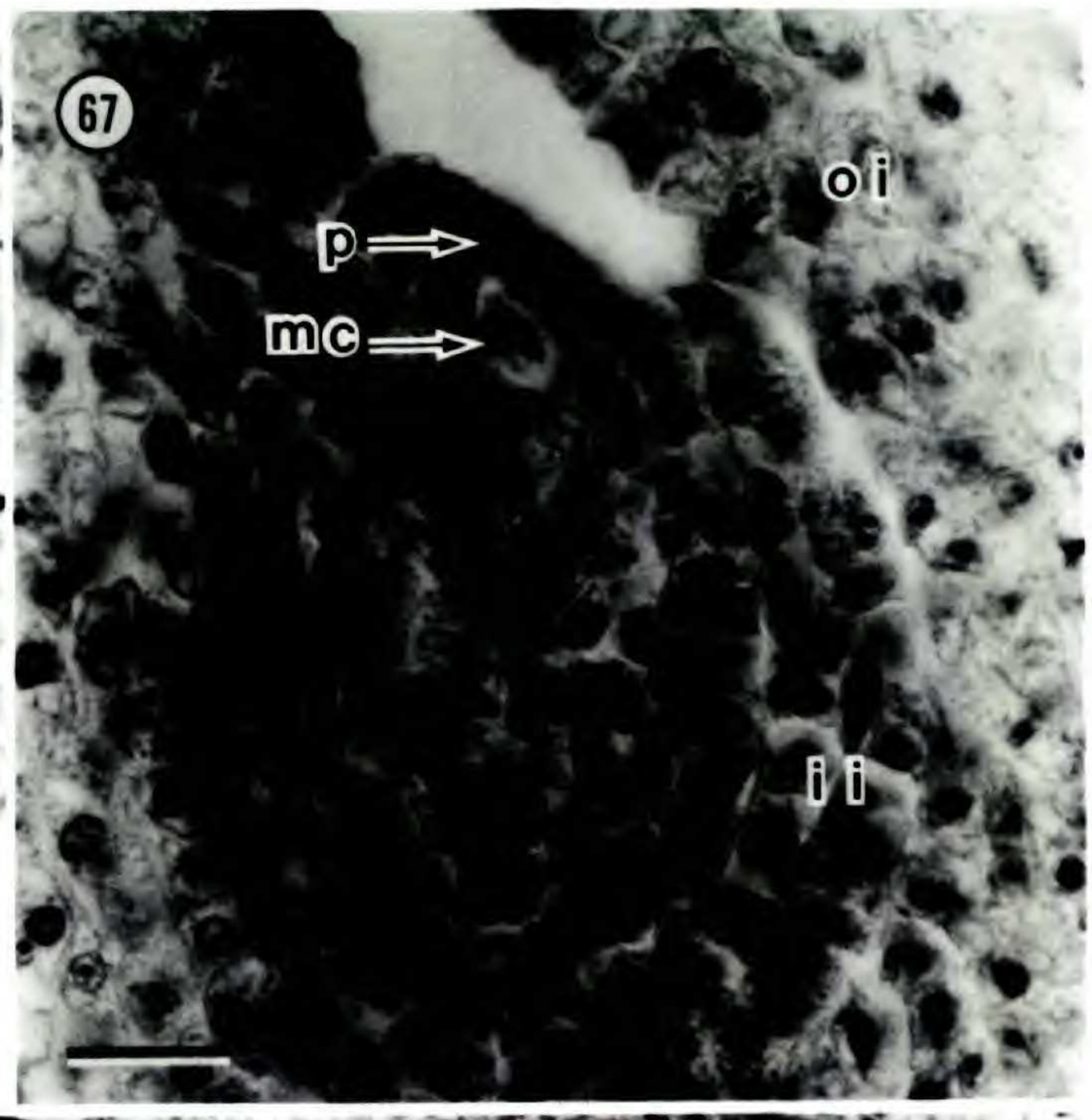


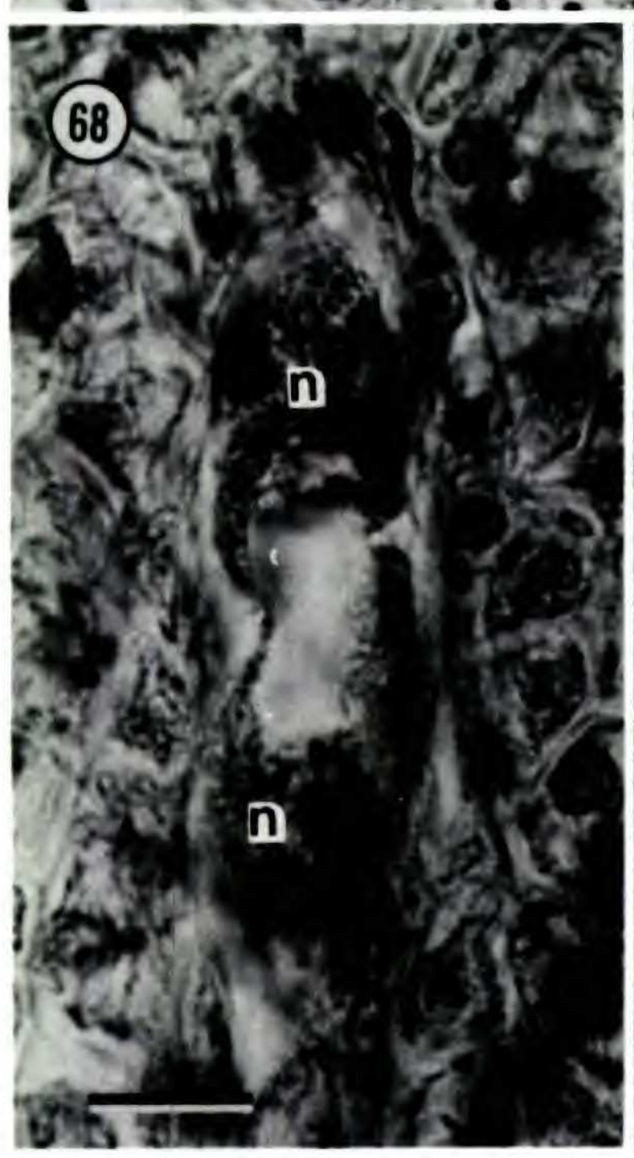


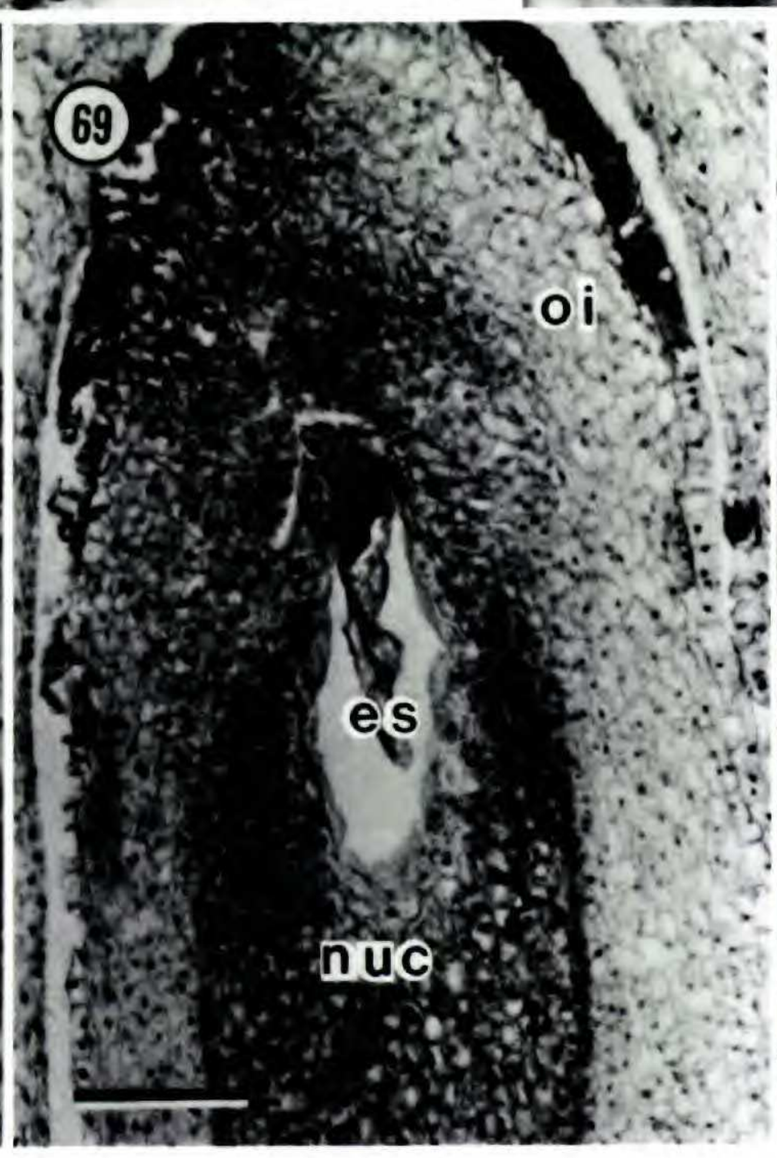


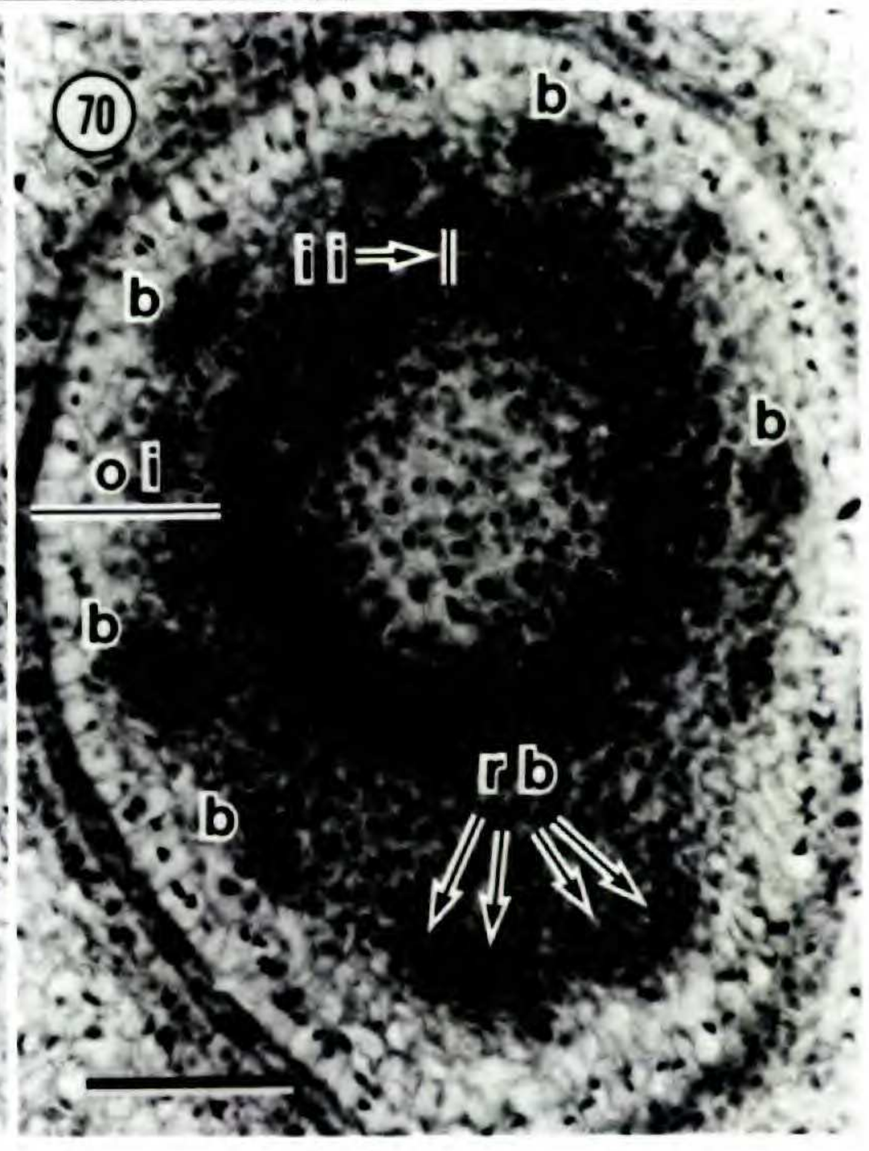
FIGURES 59–65. *Polygonanthus amazonicus*.—59. Longitudinal section of a developed anther. Arrow indicates the tapetal septum dividing a microsporangium. Bar = 50 μm.—60. Transverse section of a young anther. Bar = 10 μm.—61. TS of a mature anther at the time of dehiscence. Its wall consists of the fibrous endothecium and the epidermis. Bar = 50 μm.—62. Two-celled mature pollen at the time of shedding. Arrows indicate nuclei of the two cells. Bar = 10 μm.—63–65. Three serial sections of pollen mother cell at late prophase I showing chromosomes of $n = 7$. Seven chromosomes are numbered 1 to 7. Bars = 10 μm. t, tapetum; ep, epidermis; et, endothecium.











FIGURES 66–70. *Polygonanthus amazonicus*.—66. Longitudinal section (LS) of a young ovule. Note that the ovule is bitegmic. Bar = 50 μm.—67. Same as that shown in Figure 66, but shown at higher magnification. Note that the inner integument is two cells thick. Bar = 20 μm.—68. LS of an ovule at the 2-nucleate embryo sac stage. Bar = 10 μm.—69. LS of a mature ovule. Note that the nucellar tissue is persistent. Bar = 50 μm.—70. Transverse section of a young ovule showing that the outer integument has vascular bundles. ii, inner integument; oi, outer integument; p, primary parietal cell; mc, megaspore mother cell; n, nucleus of the embryo sac; es, embryo sac; nuc, nucellar tissue; rb, raphe bundle; b, vascular bundle.

sporium 1-celled, cutting off a primary parietal cell; embryo sac formation of the *Polygonum* type (*Anisophyllea*) or the *Allium* type (*Combretocarpus*); synergids slightly hooked; antipodals ephemeral; polar nuclei fused before fertilization (*Anisophyllea*) or not fused (*Combretocarpus*). Nucellar tissue not degenerating at least until the early stages of seed development; apical dermal nucellar cells dividing periclinally, forming a nucellar cap, chalaza without a hypostase.

Ovule unitegmic (*Anisophyllea* and *Combretocarpus*) or bitegmic (*Poga* and *Polygonanthus*); in bitegmic ovules, the inner integument two cells thick and the outer integument thicker; outer integument vascularized due to ramification of raphe bundles, but not vascularized in *Combre-*

tocarpus; micropyle formed by either the one integument or both integuments, depending on whether one or two integuments are present.

Fertilization porogamous; endosperm formation of the Nuclear type; seed exalbuminous; mode of embryogenesis not determined; embryo (potentially) dicotyledonous with a long hypocotyl, having either small cotyledons (*Combretocarpus*) or rudimentary and/or no cotyledons (*Anisophyllea* and *Poga*). Seed coat testal (*Poga*) or logically testal (*Anisophyllea* and *Combretocarpus*); mature seed coat formed by the outer epidermis alone (*Combretocarpus*), both the outer epidermis and the multiple inner layer (*Anisophyllea*), or both the multiple outer layer and the multiple inner layer (*Poga*).

Relationships with Rhizophoraceae

Although, as shown in Table 2, some of the embryological features of Anisophylleaceae are diverse, the family is consistent enough in most such characteristics to allow a more critical comparison with Rhizophoraceae than has hitherto been possible. In summary, these two families share only a few embryological features. They do agree, for example, in having a crassinucellate, bitegmic ovule and the Nuclear type of endosperm formation; but a combination of these and other shared features is widespread among many other unrelated families of the angiosperms as well.

In contrast, Anisophylleaceae differ from Rhizophoraceae in some important embryological features. First of all, in Anisophylleaceae the nucellar tissue persists until at least the early stages of seed development, whereas in Rhizophoraceae the nucellar tissue is ephemeral, disappearing completely by the time of fertilization (see Karsten, 1891, for *Rhizophora, Ceriops, Bruguiera,* and *Carallia*; Cook, 1907, for *Rhizophora*; Carey, 1934, for *Rhizophora*; Mauritzon, 1939, for *Gynotroches*; Juncosa, 1984a, 1984b, for *Bruguiera* and *Cassipourea*). Therefore in Rhizophoraceae the embryo sac borders directly on the inner integument.

Secondly, in Anisophylleaceae (*Poga* and *Polygonanthus*) the inner integument is characteristically two cells thick, whereas in Rhizophoraceae it is much thicker. Indeed, an inner integument with four to eight layers has been illustrated by Karsten (1891) for *Bruguiera, Ceriops,* and *Carallia,* by Carey (1934) for *Rhizophora,* and by Mauritzon (1939) for *Bruguiera* and *Gynotroches*. Juncosa (1984a, 1984b) described the inner integument of *Bruguiera exaristata* as initially being about 10 cells thick and that of *Cassipourea elliptica* as being about five to eight cells thick. In addition, a specialization of the innermost cell layer of the inner integument into an endothelium has been reported in some inland genera of Rhizophoraceae, including *Carallia* (Karsten, 1891), *Gynotroches* (Mauritzon, 1939), and *Cassipourea* (Juncosa, 1984a). An endothelium is never formed in Anisophylleaceae.

Thirdly, the mature seed is exalbuminous in Anisophylleaceae, but albuminous in Rhizophoraceae. The presence of abundant endosperm in mature seeds has been reported for *Rhizophora* (Cook, 1907; Carey, 1934; Juncosa, 1982), *Ceriops* (Carey, 1934), and *Cassipourea* (Juncosa, 1984a).

Some critical differences in embryo and seed coat morphology might also be added for distinguishing Anisophylleaceae from Rhizophoraceae (see Corner, 1976). However, studies on those characters in Rhizophoraceae are still too limited to allow this. Further studies on the embryology of Rhizophoraceae, including embryo and seed coat morphology, are needed to clarify the differences between this family and Anisophylleaceae.

To sum up, despite insufficient information on the embryology of Rhizophoraceae, the available data indicate that Anisophylleaceae differ significantly from them. If Anisophylleaceae were included as a tribe or subfamily, Rhizophoraceae sensu lato would be defined very broadly. With the support of exclusive occurrence of the nature of aluminum accumulation (Chenery, 1948; Kukachka & Miller, 1980); alternate, exstipulate leaves; three or four free styles (Geh & Keng, 1974); and S-type sieve-element plastids (Behnke, 1984) in Anisophylleaceae, the embryological evidence now available strongly suggests that Anisophylleaceae and Rhizophoraceae are not closely related and warrants regarding Anisophylleaceae as a distinct family.

Systematic Position of Anisophylleaceae

Cronquist (1981, 1983) has proposed assigning Anisophylleaceae to Rosales. Within Rosales, Anisophylleaceae were referred to the suborder Grossularineae, which includes six other families (Hydrangeaceae, Columelliaceae, Grossulariaceae, Greyiaceae, Bruniaceae, and Alseuosmiaceae). Of these, only Grossulariaceae have been

TABLE 2. Embryological and chromosomal data of Anisophylleaceae.

Character	*Anisophyllea*	*Combretocarpus*	*Poga*	*Polygonanthus*
Anther and microspores:				
Number of sporangia	4	4	4	4, sporangium occasionally divided by tapetal septa
Anther wall development	Basic type	Basic type	Basic type	?
Anther epidermis	Persistent	Persistent	Persistent	Persistent
Endothecium	Fibrous	Fibrous	Fibrous	Fibrous
Tapetum	Glandular	Glandular	Glandular	Glandular
Number of tapetal nuclei	2	2	2	2
Tapetal nuclear fusion	Occur	Occur	Not occur	Not occur
Cytokinesis in meiosis	Simultaneous	Simultaneous	Simultaneous	Simultaneous
Shape of microspore tetrad	Usually tetrahedral, very occasionally decussate, rarely isobilateral	Usually tetrahedral, occasionally decussate, very occasionally isobilateral	Usually tetrahedral, occasionally decussate, rarely isobilateral	?
Mature pollen	2-celled	2-celled	2-celled	2-celled
Chromosomes:				
Base number	$x = 7$	$x = 8$	$x = 7$	$x = 7$
Megagametophyte and nucellus:				
Ovule curvature	Anatropous	Anatropous	Anatropous	Anatropous
Nature of nucellus	Crassinucellate	Crassinucellate	Crassinucellate	Crassinucellate
Archesporium	1-celled	Nearly always 1-celled, very rarely 2-celled	?	?
Thickness of parietal tissue	3–5 cell-layered	1–2 cell-layered	?	?
Shape of megaspore tetrad	Linear	Linear	?	?
Functional megaspore	Chalazal cell	Chalazal cell	?	?
Pattern of embryo sac formation	*Polygonum* type	*Allium* type	?	?
Synergids	Slightly hooked	Slightly hooked	?	?
Antipodal cells	Ephemeral	Ephemeral	?	Probably ephemeral
Number of nuclei or 5 cells in mature embryo sac	5	5	?	5
Accumulation of starch grains in embryo sac	Not occur	Occur	?	Not occur
Nucellar tissue	Persistent	Persistent	Persistent	Persistent
Nucellar cap	Formed	Formed	?	Probably formed
Hypostase	Not formed	Not formed	Not formed	Not formed
Integuments:				
Number of integuments	1	1	2	2
Thickness of integuments when bitegmic	—	—	i.i. 2 cell-layered; o.i. 4–5 cell-layered	i.i. 2 cell-layered; o.i. about 5 cell-layered

TABLE 2. Continued.

Character	*Anisophyllea*	*Combretocarpus*	*Poga*	*Polygonanthus*
Vasculature	Present	Absent	Present	Present
Micropyle formation	By the only integument	By the only integument	By both integuments	By both integuments
Differentiation of endothelium	Not occur	Not occur	Not occur	Not occur
Fertilization, endosperm, and embryo:				
Path of pollen tube	Porogamous	Porogamous	?	?
Endosperm formation	Nuclear type	Nuclear type	?	?
Endosperm in mature seed	Absent	Absent	Absent	?
Embryogenesis	?	?	?	?
Embryo in mature seed	Cotyledonous or not cotyledonous	Cotyledonous	Not cotyledonous	?
Size of cotyledons when present	Very small (rudimentary)	Small	—	?
Mature seed and seed coat:				
Shape of seed	Narrow–cylindrical (*A. disticha*); ovoid or elliptical (*A.* sp.)	Linear	Ovoid and slightly suppressed toward raphe-antiraphe direction	?
Size of seed	13.0–13.6 mm long and 3.8–4.0 mm diam. (*A. disticha*); 13.0–13.8 mm long and 6.0–6.4 mm diam (*A.* sp.)	9.5–10.4 mm long and 1.2–1.3 mm diam.	20.5–22.5 mm long, 12.0–13.5 mm wide	?
Tegmen	—	—	Ephemeral	?
Whole thickness of seed coat (SC)	26–31 cell-layered	1 cell-layer	17–20 cell-layered	?
Thickness of inner layer of SC	25–30 cell-layered	—	7–10 cell-layered	?
Thickness of outer layer of SC	1 cell-layer	1 cell-layer	About 10 cell-layered	?

i.i., inner integument; o.i., outer integument.

relatively well studied embryologically, whereas the others have been studied little or not at all in this respect. Anisophylleaceae differ from all Grossularineae in having exalbuminous seeds (Cronquist, 1981). On the other hand, Anisophylleaceae resemble Grossulariaceae (principally *Ribes*, from which most data are available) in nearly all features of anther and microspore development; in their anatropous, bitegmic, and crassinucellate ovule; *Polygonum*-type embryo sac; ephemeral antipodal cells; inner integument with two cell layers (see Davis, 1966; Corner, 1976; Cronquist, 1981, for data on Grossulariaceae). However, Anisophylleaceae differ from Grossulariaceae in several embryological features. For example, the tapetal cell is basically 2-nucleate in Anisophylleaceae, but multinucleate in Grossulariaceae; the integument is vascularized in Anisophylleaceae, but not vascularized in Grossulariaceae; endosperm formation is of the Nuclear type in Anisophylleaceae, but of the Cellular or the Helobial type in Grossulariaceae; the tegmen is ephemeral in Anisophylleaceae (*Poga*), but persists in Grossulariaceae; the seeds are non-arillate in Anisophylleaceae, but arillate in Grossulariaceae (see Netolitzky, 1926; Davis, 1966; Corner, 1976, for data on Grossulariaceae). Therefore it seems that available embryological evidence neither supports nor denies a close relationship between Anisophylleaceae and Grossularineae (Rosales).

In contrast, Dahlgren (1983) placed Aniso-

phylleaceae in the Cornales, which comprise 27 families including Hydrangeaceae (and five families whose position is uncertain; see also Dahlgren & Thorne, 1984). Of 27 families, nine have either not been studied embryologically, or have been studied only to a very limited degree. Of the 18 remaining families, nearly all share a unitegmic ovule, ephemeral nucellar tissue, endothelium, Cellular type of endosperm formation, and albuminous seed. The Cornales thus seem to be very well defined by a combination of those shared embryological features. Anisophylleaceae, which lack any of those characteristic embryological features of the Cornales (almost certainly unitegmic ovule in *Anisophyllea* and *Combretocarpus*), seem clearly distinct from the Cornalean families and do not belong in that order.

We would rather suggest Myrtalean affinities for Anisophylleaceae. Embryologically, Anisophylleaceae agree almost completely with Myrtales, and in fact share the eight ordinal embryological features (see Tobe & Raven, 1983, 1984): 1) anther tapetum glandular, 2) ovule crassinucellate, 3) inner integument two cells thick, 4) micropyle formed by both integuments, 5) antipodal cells ephemeral, 6) endosperm formation—Nuclear type, 7) seed exalbuminous, and 8) mature pollen 2-celled. One might point out a fusion of tapetal nuclei (in *Combretocarpus* and *Anisophyllea*), formation of the nucellar cap, and testal seed as features distinguishing Anisophylleaceae from Myrtales. However, nuclear fusion in the tapetal cells is undoubtedly a secondary characteristsic that evolved in two genera of Anisophylleaceae. Indeed *Poga* and *Polygonanthus,* both of which have many primitive features, as will be discussed later, have unfused tapetal nuclei. The nucellar cap, which is formed by derivatives of the apical nucellar dermal cells, is commonly observed in Combretaceae (Myrtales; Venkateswarlu & Rao, 1972). A seed coat lacking a tegmen is frequent in Melastomataceae (Myrtales; Corner, 1976). Anisophylleaceae may differ from Myrtales in having embryos with reduced or rudimentary cotyledons and a long hypocotyl. Such an embryo morphology seems to result in hypogeal germination, which is reported in at least *Anisophyllea disticha* (Geh & Keng, 1974). The peculiar embryo morphology and germination habit may suggest a specialized position of Anisophylleaceae. However, embryos devoid of cotyledons are recorded in many unrelated families, a majority of them growing in ecologically specialized habitats (Natesh & Rau, 1984, review). Study of embryogenesis and organogenesis in seeds through germination seems to be needed for the elucidation of the ecological significance of such specialized embryos in Anisophylleaceae. Except for the difference in embryo morphology, there seems to be essentially a perfect correspondence in embryological features between Anisophylleaceae and Myrtales.

Viewing other reproductive and vegetative character states, Anisophylleaceae lack both the intraxylary phloem and the vestured pits, which are regarded as characteristic features defining the Myrtales (Van Vliet, 1976; Van Vliet & Baas, 1984; Dahlgren & Thorne, 1984). However, the occurrence of S-type sieve-element plastids in *Anisophyllea* and *Combretocarpus,* in contrast with the P-type plastids in Rhizophoraceae, agrees with Myrtales (Behnke, 1982, 1984). Tricolporate pollen morphology in Anisophylleaceae (as well as in Rhizophoraceae) is of the basic type found in the Myrtales (Erdtman, 1966; see also Dahlgren & Thorne, 1984). Aluminum accumulation characteristic of Anisophylleaceae (unknown in Rhizophoraceae) is known to occur in Crypteroniaceae and Melastomataceae of Myrtales (Chenery, 1948; Kukachka & Miller, 1980).

Thus, considering a considerable number of coincidences (in reproductive anatomy) in contrast with a limited number of differences (in wood anatomy), in conjunction with support by sieve-element plastid type, palynology, and aluminum accumulation, it seems that Anisophylleaceae are closely related to Myrtales. Depending on how we interpret the lack of intraxylary phloem and vestured pits in Anisophylleaceae, it might even be justifiable to place Anisophylleaceae in the Myrtales. According to Van Vliet and Baas (1984), the combined occurrence of intraxylary phloem and vestured pits is very restricted in the dicotyledons; in fact, except for the Myrtales, this combination is found only in part of the Gentianales, Thymelaeales, Polygalales, and Polygonales. Outside these orders, either one of these features (but not both) sporadically occurs in many different groups of dicotyledons (see Van Vliet & Baas, 1985: 784, fig. 1). Only a few orders are characterized by consistent possession of one or both of those two wood anatomical features.Therefore it does not seem that the lack of intraxylary phloem and vestured pits in Anisophylleaceae necessarily precludes a possibility of close affinity with Myrtales. Based on total evidence now available, we

would suggest that Anisophylleaceae be placed near Myrtales. Perhaps Anisophylleaceae represent one of the groups that diverged from a common ancestral stock with Myrtales and then spread widely. The position of Anisophylleaceae will be evaluated better as the Rosiflorae or the Rosales, which are considered phylogenetically basic in position with respect to Myrtales, are understood better embryologically.

Interrelationships and Evolution of the Genera

Because of many shared embryological features, as shown in Table 2, as well as of shared vegetative and some other shared reproductive features (see also Geh & Keng, 1974; Van Vliet, 1976), there is no doubt that *Anisophyllea, Combretocarpus, Poga,* and *Polygonanthus* are monophyletic. Despite the lack of data about several features in *Poga* and *Polygonanthus,* the available embryological data are now enough to allow us to compare all four genera.

Of these, *Combretocarpus* is the most distinct. It has a gametic chromosome number of $n = 8$, *Allium* type embryo sac, nonvascularized integument, starch grains in the embryo sac, cotyledonous embryo, and thin mature seed coat one cell layer thick. In contrast, *Anisophyllea, Poga,* and *Polygonanthus* have a chromosome number of $x = 7$, *Polygonum* type embryo sac (unknown in *Poga* and *Polygonanthus*), vascularized integument, no starch grains in the embryo sac, noncotyledonous embryo (unknown in *Polygonanthus*), and thick mature seed coat (unknown in *Polygonanthus*). *Combretocarpus* agrees with *Anisophyllea* only in having fused tapetal nuclei and a unitegmic ovule. On the contrary, *Anisophyllea* differs from *Poga* and *Polygonanthus* in sharing neither bitegmic ovules nor distinct tapetal nuclei as well as in not sharing a multiple outer layer in the mature seed coat (though uncertain in *Polygonanthus*). *Polygonanthus* differs from *Poga* only in its occasionally divided microsporogenous tissue. Except for this, there is no essential difference between *Poga* and *Polygonanthus,* as far as the data available are concerned.

In order to clarify phylogenetic interrelationships of the genera, it seems necessary to evaluate each of the characters showing differences between them. Therefore, as an attempt, we evaluated embryological character states of Anisophylleaceae following Eldredge and Cracraft (1980) as regards principles of analyzing methods of character state similarities (i.e., synapomorphies or symplesiomorphies). Of the embryological features, the *Polygonum* type embryo sac formation (*Anisophyllea*) that is characteristic of a majority of the dicotyledons (Davis, 1966) is undoubtedly primitive to the *Allium* type manner (*Combretocarpus*), and also bitegmy (*Poga* and *Polygonanthus*) is primitive to the unitegmy (*Anisophyllea* and *Combretocarpus*; Bouman, 1984).

Concerning the vasculature of the integument, there is no consensus regarding whether or not the vascularized integument represents an archaic condition. Bouman (1984) suggested that there seems to be a general relation between the size of ovules or seeds and the degree of vascularization. As far as Anisophylleaceae are concerned, the vascularized integument or testa (*Anisophyllea, Poga,* and *Polygonanthus*) is probably primitive (symplesiomorphous) compared to the nonvascularized one (*Combretocarpus*). *Combretocarpus* has multiple vascular bundles in the raphe of the mature seed (see Fig. 42). This vascular condition in *Combretocarpus* is probably derived from the condition seen in the three other genera by suppression of vascular extension into the integument, because *Combretocarpus* has the thin integument that eventually becomes the one cell-layered seed coat at maturity. In this connection, the thick mature seed coat or testa is probably primitive (symplesiomorphous) to the thin, one cell-layered mature seed coat. Compared with *Poga, Anisophyllea* lacks a hypodermal tissue in the thick multiple outer layer of *Poga*; *Combretocarpus* lacks both the multiple inner layer and the hypodermal tissue of the multiple outer layer. Corner (1976, vol. 1: 57) has considered the limitation of a multiple mechanical tissue (probably like that of *Poga*) into one cell-layered as one of specialization trends of seed coat. Following Corner, we may be able to postulate that the successive or simultaneous reduction of the multiple inner layer and the hypodermal tissue of the multiple outer layer had occurred in the seed coat evolution of Anisophylleaceae so that only the epidermis was persistent, as in *Combretocarpus.* Although we did not observe the anatomy of the testa of *Polygonanthus,* it was confirmed that the (outer) integument shows a secondary multiplication, a condition that is clearly different from that in *Combretocarpus.* Therefore it seems very likely that *Polygonanthus* would form a mature seed with as thick a testa as in *Poga.*

TABLE 3. Evolutionary trend of karyological and some embryological characters in Anisophylleaceae.

Characters	Plesiomorphy	Apomorphy
1. Number of integuments	2	1
2. Tapetal nuclei	Not fused	Fused
3. "Outer layer" of the "thick" seed coat (thickness)	Thick	Thin, 1 cell thick
4. Chromosome number	$n = 7$	$n = 8$
5. Pattern of embryo sac formation	*Polygonum* type	*Allium* type
6. "Inner layer" of the mature seed coat	Present	Absent
7. Accumulation of starch grains in developing embryo sac	Absent	Present
8. Vasculature of integuments	Present	Absent

The accumulation of starch grains in the developing embryo sac (*Combretocarpus*) is known to occur in many unrelated families of dicotyledons (see Davis, 1966). Even within a family, however, their occurrence is in general restricted to certain genera. Therefore the occurrence of starch grains seems to have been acquired secondarily by particular groups in many unrelated families, probably because of the necessity of different metabolic activity during megagametogenesis.

Embryos with moderately developed cotyledons are almost universal among dicotyledons and therefore must be primary. On the contrary, because of their restricted occurrence (see Natesh & Ram, 1984), embryos lacking cotyledons seem to be secondary in the evolutionary trend. In this respect, it might be interpreted that an embryo with small cotyledons (*Combretocarpus*) seems less specialized than that which lacks cotyledons, or at the most has rudimentary ones (*Anisophyllea* and *Poga*). Such differences in the degree of size reduction of cotyledons, however, may be a matter of degree, because it seems to be more fundamentally important that Anisophylleaceae share a conspicuous hypocotyl, a truly significant and unusual feature.

It may be difficult to determine whether the chromosome base number of Anisophylleaceae is $n = 7$ or 8. Noticeably $n = 8$ occurs only in *Combretocarpus*, a genus that is furnished with many advanced and fewer primitive character states as discussed above. In contrast, $n = 7$ is shared by *Anisophyllea*, *Poga*, and *Polygonanthus*, all of which—particularly the latter two—retain a combination of primitive character states. Thus it seems likely that $n = 7$ is the base number of the family, and $n = 8$ the derived.

The fusion of tapetal nuclei (*Anisophyllea* and *Combretocarpus*) certainly seems secondary to the condition in which the two nuclei remain distinct (*Poga* and *Polygonanthus*), as discussed earlier. These nuclei also remain distinct in most Myrtales (Tobe & Raven, 1983).

The results of our evaluation of these character states are summarized in Table 3. On this basis, we constructed a cladogram illustrating the evolutionary interrelationships of the genera (Fig. 71). The cladogram indicates that the proto-Anisophylleaceae, a hypothetical ancestor of the family, had nearly all of the embryological features that are presently retained by *Poga* and *Polygonanthus*: a chromosome number of $n = 7$, *Polygonum* type embryo sac (although actually uncertain in *Poga* and *Polygonanthus*), bitegmy, vascularized integument, thick seed coat consisting of a multiple inner layer and a multiple outer layer, no starch grain accumulation during megagametogenesis, and non-fused tapetal nuclei. An ancestral evolutionary line seems to have diverged into two main branches: one leading to *Combretocarpus* and *Anisophyllea*, and the other leading to *Poga* and *Polygonanthus*. In the former branch, the ovule became unitegmic; tapetal nuclei fusion has been generalized, and the thickness of the multiple outer layer was reduced into one cell layer (i.e., the outer epidermis); all three characters are synapomorphies common to *Combretocarpus* and *Anisophyllea*. This branch further diverged into two branchlets: one leading to *Combretocarpus*, and the other leading to *Anisophyllea*. In the branchlet leading to *Combretocarpus*, chromosome base number changed to $n = 8$; the *Allium* type embryo sac and unitegmy were derived; complete reduction of seed coat tissue except for the outer epidermis (i.e., of both a multiple inner layer and the hypodermal tissue of the original thick seed coat) and reduction of integumentary vasculature occurred nearly simultaneously; and accumulation of starch grains during megagametogenesis was generalized. With respect to embryological characteristics, no striking change has occurred in the other branchlet

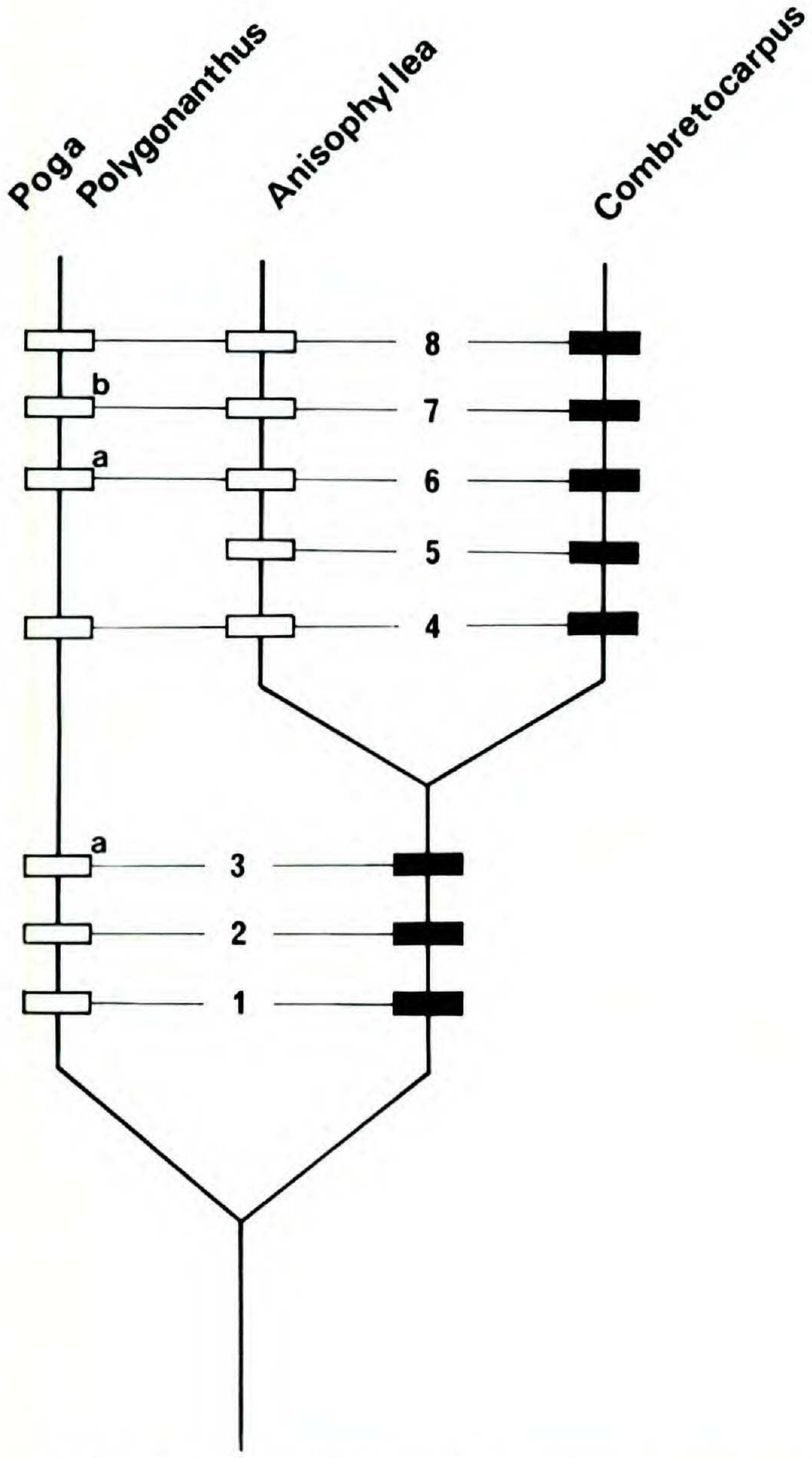


FIGURE 71. A cladogram illustrating postulated evolutionary interrelationships of the genera of Anisophylleaceae. Corresponding characters numbered 1 to 8 and their evolutionary states are shown in Table 3. Shaded rectangles indicate apomorphies. [a]Character state uncertain in *Polygonanthus*; [b]Character state uncertain in *Poga*.

leading to *Anisophyllea*, or in the other main evolutionary line leading to *Poga* and *Polygonanthus*.

Consequently, *Combretocarpus* differs substantially from the three other genera and is apparently the most specialized member of the family. In contrast, most plesiomorphies are referred to *Poga* and *Polygonanthus*, which both retain a combination of primitive embryological character states common to the ancestor of Anisophylleaceae. *Anisophyllea* stands in a more or less intermediate position between *Combretocarpus*, on the one hand, and the group comprising *Poga* and *Polygonanthus*, on the other. Indeed *Anisophyllea* shares most of its archaic features with *Poga* and *Polygonanthus* but shares its apomorphies, including unitegmy and tapetal nuclear fusion, with *Combretocarpus*.

Phylogenetic interpretations of the infrafamilial relationships as discussed above are primarily based on embryological character state evaluation and chromosome number. The cladogram shown in Figure 71 is to be regarded as limited in this respect and provisional. The evolutionary trend in certain characters (such as chromosome number and seed coat structure) might be the opposite of what we have proposed, and certain characters (such as tapetal nuclear condition) might be of much less fundamental significance than others. Earlier studies on Anisophylleaceae never discussed the interrelationships of these genera at much length, however, because the interest earlier was primarily directed toward whether or not *Polygonanthus* was closely related to the other three genera (for instance, Pires & Rodrigues, 1971) or whether or not Anisophylleaceae ("Anisophylleae") should be excluded from Rhizophoraceae (for instance, Van Vliet, 1976). We now regard both of these questions as definitively solved and hope that our phylogenetic diagram will stimulate further research on the family from various other points of view and will thus be improved as a result of these investigations.

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